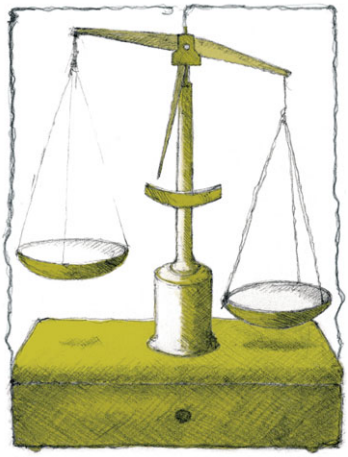
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

SLEEP REGULATION



SLEEP CONCERNS: IS NAPPING LINKED TO BODY CONDITION?

After an ample meal, all of us are familiar with the sometimes irresistible urge to take a nap. However, obeying this urge while forking down dinner, lunch or tea could quickly turn into an inconvenient complaint, generally diagnosed as 'excessive daytime sleepiness'. This sort of illness represents not only a growing health problem associated with our hectic lifestyles but also offers new insights into the link between sleep and obesity.

To specifically address the issue of sleep regulation and body condition in mammals, Joe Jenkins and his colleagues from the Department of Psychiatry in Pennsylvania, USA performed an appetising experiment on mice. They measured electroencephalogram (EEG) and electromyography (EMG) activity in the brains of black mice while they were awake and during sleep. Before starting the experiment, the team recorded baseline sleep patterns while feeding regular lab chow to all the mice. Next, they divided the mice into two groups; one that was fattened with high fat food, and another, the control group of mice, which kept their figures by ingesting normal food. As the scientists were interested in the sleep behavior of obese and thin mice, they continuously monitored the animal's brain activity while asleep, and awake over 2, 4 and 6 week intervals. The animal's sleep and awake states were scored by quantifying brain activity wavelengths from the EEG and EMG recordings and attributing them either to non-rapid eye movement sleep (NREMS), rapid eye movement sleep (REMS), or to the awake behavioural state. In addition to that, Jenkins and his team measured the animal's food intake and body mass during the course of the experiment.

Consistent with observations in well-fed humans, the researchers found that in the experimentally fattened mice, the animal's sleepiness increased in parallel with their weight gain. Simultaneously, their wakefulness declined after 2, 4 and 6 weeks of gorging. Interestingly, the mice's REMS and NREMS sleep patterns were affected differently by weight gain; episodes of NREMS significantly increased, whereas their REMS remained unaltered. By correlating the increase of NREMS with both the increase in body weight and energy intake, Jenkins and his co-workers found out that the weight gain itself is responsible for increased sleepiness, not the net food intake. They explain that the underlying metabolic process for this finding remains to be uncovered and propose taking a closer look at the expression of inflammatory cytokines such as tumor necrosis factor (TNF) and interleukin-6 (IL-6). As obesity is a chronic inflammatory state, these two cytokines, which either circulate in plasma (TNF) or are found in fat (IL-6), are both elevated in obese individuals, and may regulate sleep or are possibly involved in sleep disorders. Altogether, Jenkins' study indicates that obese animals experience an increased pressure to sleep resulting in a reduction in their wakefulness, especially during the active phase of the day. So those of us who dreamt of catching forty winks before reading this are advised to sleep on it before taking that snooze.

10.1242/jeb.02222

Jenkins, J. B., Omori, T., Guan, Z., Vgontzas, A. N., Bixler, E. O. and Fang, J. (2006). Sleep is increased in mice with obesity induced by high-fat food. *Physiol. Behav.* doi:10.1016/j.physbeh.2005.10.010

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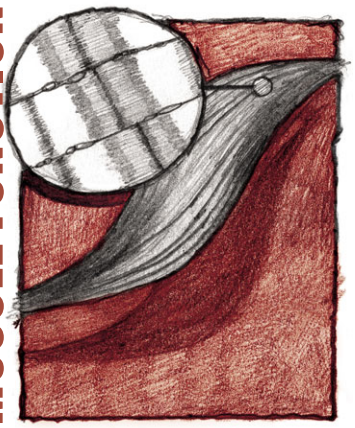
MUSCLE FUNCTION



NOT YOUR TYPICAL BABY'S RATTLE

If I heard the sound of a rattlesnake's rattle, I'd run away as quickly as possible. Surprisingly, some of my colleagues not only lack this reflex, but go out of their way to elicit and listen closely to snake rattling. In doing so, they are learning some interesting things about the development of this behavior and the underlying tail musculature that drives it.

Within the world of muscles, those that shake the rattlesnake's tail are an impressive bunch, able to contract at very high frequencies for long durations. Recently, Brad Moon and Alexa Tullis observed that the frequency with which a snake shakes its rattle changes over ontogeny, increasing considerably between newborns and adults. This was particularly intriguing given Moon's previous work documenting a small decrease in contractile frequency between medium and large-sized adults. Apparently, contractile performance first increases, then decreases, in relation to body size in rattlesnakes. Not content with just documenting this pattern, Moon and Tullis then set out to determine the underlying causes.

They hypothesized that two disparate mechanisms were at work, one biochemical, and the other mechanical. They believed that shifts in muscle metabolic capacity likely explained the initial increase in contractile performance between newborns and adults. In contrast, they proposed that the subsequent decrease in performance as animals continued to grow was linked to simple mechanics. Bigger snakes have a more ossified, and thus disproportionately heavier, bone in the base of the rattle, and it is harder for the tail muscle to move these larger sound makers.

To test their metabolic hypothesis, the researchers assayed the activity of citrate synthase (CS), an important enzyme for aerobic metabolism, in tail shaker muscle from snakes ranging between 11 and 911 g. CS activity was significantly and positively correlated with contraction frequency, i.e., CS activity changed with body size in a manner similar to contractile frequency, first increasing rapidly then slowly decreasing with body size. To test their biomechanical hypothesis, Moon and Tullis measured how the mass of the rattle, using its first segment as a proxy, changed with growth, and compared that to how the cross-sectional area (CSA) of the tail shaker muscle changed. Because muscle force is proportional to its CSA, rattle mass multiplied by rattle acceleration should be proportional to tail shaker muscle CSA, and to maintain a constant rattle acceleration would require rattle mass to increase proportionately with muscle CSA. In fact, rattle mass increases faster than muscle CSA as snakes grow, implying that rattle acceleration must decrease with size. Because the distance a rattle moves during shaking doesn't decrease with body size, this reduced acceleration necessitates a decrease in rattle frequency.

In summary, the increase in rattle frequency found between newborns and adults can be explained by a concomitant increase in tail shaker muscle aerobic capacity (i.e., CS activity). A subsequent small reduction in this capacity, in addition to a mismatch between the strength of the tail muscle and the mass of the rattle it must shake, underlie the subtle decrease in rattle frequency observed as adult snakes continue to grow. See what you could learn if you were brave enough to stick around and listen carefully to a diamondback's rattle…

10.1242/jeb.02218

Moon, B. R. and Tullis, A. (2006). The ontogeny of contractile performance and metabolic capacity in a high-frequency muscle. *Physiol. Biochem. Zool.* **79**, 20-30.

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PAIR BONDING

FIRST AND ONLY LOVE

Monogamy is defined as the condition of having only one mate. But have you ever wondered what's happening at the neuronal level when we choose that special someone? In a recent paper in *Nature Neuroscience*, Brandon Aragona and colleagues show that dopamine activity in the nucleus accumbens, a structure in the brain known to be involved in controlling social interactions, is necessary for pair bonding behaviour in the monogamous prairie vole.

The neurobiology underlying both pair bond formation and maintenance is poorly understood. However, the prairie vole has proven to be an excellent model for addressing these questions because they easily form pair bonds and the behaviour they emit upon forming a pair bond is readily quantifiable. A pair bond is established when a male vole prefers a particular mating partner **and** potential female mates are subsequently rejected aggressively. It is known that dopamine transmission in the nucleus accumbens *via* the D2-like receptors (D2 receptors) and D1-like receptors (D1 receptors) mediates both approach and avoidance behaviours and thus the authors focused on this system to study pair bond formation and maintenance.

The team measured partner preference in a male prairie vole by recording the duration of side-by-side contact it made with a particular female and when it displayed selective aggression by the number of attacks and aggressive postures the male vole struck toward other female voles. Activation of the D2-like receptors, but not D1-like receptors, in the nucleus accumbens had previously been shown to be important in forming a pair bond. However, it was unclear where in the nucleus accumbens this signaling occurs, or, how a pair bond is maintained. By injecting a D2 receptor agonist into specific

subregions of the nucleus accumbens, the authors demonstrated that dopamine activity via the D2 receptors in the rostral shell of the nucleus accumbens is necessary to form a pair bond.

Knowing that a long-lasting change in behaviour is associated with changes in synaptic connectivity, the authors hypothesized that a reorganization of the dopamine signaling system in the nucleus accumbens was responsible for pair bond maintenance and found an increase in D1 receptors in the nucleus accumbens 2 weeks after forming a partner preference, which was associated with increased aggression toward other females. Since dopamine activity *via* the D1 receptors blocks pair bond formation, the authors hypothesized that the upregulation of these receptors prevents the formation of a second pair bond, thus promoting stable maintenance of the initial pair bond.

This study clearly demonstrates that dopamine activity in the nucleus accumbens regulates monogamous pair bonding. Initially, in a sexually naive male, dopaminergic signaling, primarily *via* D2 receptors, facilitates a partner preference, a positive association. Subsequent to forming a pair bond, dopaminergic signaling, now primarily *via* D1 receptors, indicates an aversive stimulus thus increasing aggression towards other females and preventing a second pair bond. Dopamine signaling in the nucleus accumbens is not only important for pair bonding but also in drug addiction. Thus, the data here also supports the hypothesis that drugs of abuse target neural systems that evolved to mediate adaptive behaviours such as social bonding.

10.1242/jeb.02221

Aragona, B. J., Liu, Y., Yu, Y. J., Curtis, J. T., Detwiler, J. M., Insel, T. R. and Wang, Z. (2006). Nucleus accumbens dopamine differentially mediates the formation and maintenance of monogamous pair bonds. *Nature Neurosci.* **9**, 133-139.

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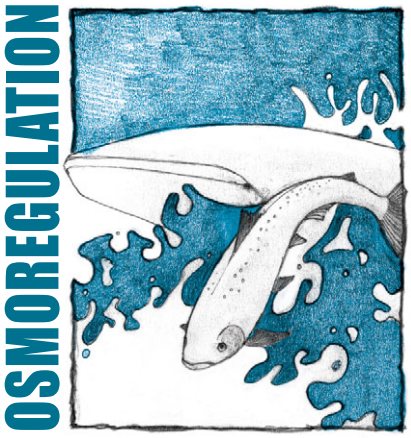

FISHY TARGETS FOR MELATONIN

Melatonin, probably best known for its influence on circadian and seasonal rhythms within vertebrates, is actually involved in many other physiological processes. Derived from the neurochemical serotonin, melatonin is a small molecule that can penetrate all tissues easily. For this reason, it would be expected that melatonin would be present in many different organs at concentrations similar to those in plasma, potentially with the same diurnal rhythm. However, if melatonin is found in tissues at concentrations higher than this, it may suggest an active uptake and/or local synthesis of melatonin and a potential role for the molecule within this organ. Physiological effects of melatonin are mediated *via* high-affinity G-protein coupled receptors, which can be identified *in vitro* by autoradiography and conventional binding assays using 2-[^{125}I]Iodomelatonin, a radiolabelled marker for melatonin. In the present study, Ewa Kulczykowska, Richard Balment and their group of researchers decided to examine for the first time the potential involvement of melatonin in fish osmoregulatory tissues. They set out to determine the concentration of melatonin as well as the distribution of the specific binding of 2-[^{125}I]Iodomelatonin, which would indicate the location of melatonin receptors, in the kidney, gill and intestine of rainbow trout, flounder and seabream.

The fish were held under a controlled 12 h light:12 h dark illumination regime for at least a period of three days before experimentation. After this acclimation period, plasma melatonin in the trout was measured by high performance liquid chromatography (HPLC), whereas plasma melatonin in seabream and flounder, as well as tissue melatonin levels in all three fish, were assayed using a total melatonin radioimmunoassay. Consistent with findings throughout the animal kingdom, the plasma melatonin levels in trout, flounder and seabream were significantly higher at night than during the day. However, melatonin levels remained unchanged in almost all of the tissues in the three species over a 24 hour cycle. They did not show the diurnal variation that had been found in the fish's plasma, suggesting that levels within these tissues are regulated independently of plasma concentrations. The team also demonstrated 2-[^{125}I]Iodomelatonin binding in the kidney, gill and small intestine and that iodomelatonin binding did not fluctuate during the day, suggesting that the action of melatonin in osmoregulatory tissues is not photoperiod-dependent. Furthermore, GTPγS, a compound specific for G-protein receptors, added to the incubation medium significantly reduced the binding of 2-[^{125}I]Iodomelatonin in gill and intestine of trout and flounder by 30–50%, strongly suggesting that the 2-[^{125}I]Iodomelatonin binding sites are associated with a G-protein in these tissues.

This study is the first to provide evidence for the presence of melatonin binding in fish osmoregulatory tissues. Up until now, reports of melatonin binding in fish have been limited to the brain and the heart. Interestingly, in all three species, the concentration of melatonin in the intestine was significantly higher than those in the plasma, with the greatest difference occurring during the day when plasma levels were at their lowest. This suggests that in addition to the pineal gland and the retina, the intestine may be an important source of melatonin, as seen in other vertebrates. Its high melatonin content together with its G-protein mediated iodomelatonin binding suggests that melatonin in the intestine in particular may play an important physiological role in fish, more so than in the gill and kidney. What exactly that role is, has yet to be determined.

10.1242/jeb.02219

Kulczykowska, E., Kalamarz, H., Warne, J. M. and Balment, R. J. (2006). Day-night specific binding of 2-[^{125}I]Iodomelatonin and melatonin content in gill, small intestine and kidney of three fish species. *J. Comp. Physiol. B* DOI 10.1007/s00360-005-0049-4

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HEAT UP AND SPEED UP

Watch an athlete preparing for a race and the one thing they focus on is their warm-up routine. This is because muscle performance is better at warm temperatures. For example increased temperature improves oxygen unloading from hemoglobin and myoglobin and it may also reduce the risk of strains and pulls. Elevated temperature also increases nervous signal transduction and a warmed muscle contracts more vigorously and relaxes more quickly due to increases in the crossbridge cyling and ATP turnover rates. The benefits of increased ATP turnover are most pronounced during intense sprints, when the muscle works close to its maximal limits for a short time. It is, therefore, a paradox that the type I muscle fibres, which are associated with endurance performance, seem to be more responsive to elevated ATP turnover and power output at raised temperatures, than the type IIA fibres associated with sprinting. To investigate this Stuart Gray and his colleagues from University of Strathclyde and Aalborg University investigated the importance of elevated muscle temperature for performance during a 6-second bicycle sprint. In particular they examined the increased scope for ATP turnover and the increased velocity of muscle fibre activation in sprinting warm muscle.

All of the participants in the study performed the same sprint test at both normal and elevated muscle temperatures. But instead of warming the test person's muscles with exercise, they raised their muscle temperature by placing the cyclists in a warm bath. During both 'normal' and elevated temperature cycle sprints, the team measured the athletes' power outputs and pedal rates. In addition the scientists took a muscle biopsy from each of the sportsmen immediately before and after the sprint test in order to assess the ATP turnover. Finally, the muscle fibre conduction velocity was measured with a multi channel surface EMG recording from the thigh muscle.

The group found that the ~3°C increase in muscle temperature significantly increased ATP turnover and that this increase was associated with an increase in muscle fibre conduction velocity. This indicated that the increased energy turnover in warmed muscles was linked to the faster activation of the muscle fibres, and warming was also associated with an in increase in pedal rate and in total power output so that the maximal power output was ~20% higher when the participants had warm muscles. In contrast to some previous studies on maximal sprint performance, Gray and colleagues conducted the sprint test so that the participants were allowed to increase the sprint speed (pedal rate) instead of increasing the load against which they were working. Under these conditions, which are probably more realistic in terms of real sprints, the authors found that the increase in power output correlated best with the amount of Type IIA 'sprint' muscle fibres. Thus, when the fast muscles fibres are allowed to work fast they also benefit considerably from the increased temperature and this result resolves the apparent paradox where it was thought that it was mainly the 'endurance' fibres that benefited from increased ATP-turnover.

Even though an increased ATP turnover and power output at elevated temperatures is not a novel finding in itself, the study by Gray and his team re-emphasises the importance that a few degrees rise in muscle temperature can have for the tissue's function. Considering that sprint competitions are often decided by only a few hundredths of a second it certainly seems to be important to warm up well in order to get ahead.

10.1242/jeb.02220

Gray, S. R., De Vito, G., Nimmo, M. A., Farina, D. and Ferguson, R. A. (2006). Skeletal muscle ATP turnover and muscle fiber conduction velocity are elevated at higher muscle temperatures during maximal power output development in humans. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **290,** R376-R382.

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