Exercise and Brain Neurotransmission

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Summary

Physical exercise influences the central dopaminergic, noradrenergic and serotonergic systems. A number of studies have examined brain noradrenaline (norepinephrine), serotonin (5-hydroxytryptamine; 5-HT) and dopamine with exercise. Although there are great discrepancies in experimental protocols, the results indicate that there is evidence in favour of changes in synthesis and metabolism of monoamines during exercise.

There is a possibility that the interactions between brain neurotransmitters and their specific receptors could play a role in the onset of fatigue during prolonged exercise. The data on the effects of branched chain amino acid (BCAA) supplementation and 'central fatigue' seem to be conflicting, although recent studies suggest that BCAA supplementation has no influence on endurance performance.

There are numerous levels at which central neurotransmitters can affect motor behaviour, from sensory perception, and sensory-motor integration, to motor effector mechanisms. However, the crucial point is whether or not the changes in neurotransmitter levels trigger or reflect changes in monoamine release. Until recently most studies were done on homogenised tissue, which gives no indication of the dynamic release of neurotransmitters in the extracellular space of living organisms.

Recently, new techniques such as microdialysis and voltammetry were introduced to measure in vivo release of neurotransmitters. Microdialysis can collect virtually any substance from the brain of a freely moving animal with a limited
amount of tissue trauma. This method allows measurement of local neurotransmitter release during on-going behavioural changes such as exercise.

The results of the first studies using these methods indicate that the release of most neurotransmitters is influenced by exercise. Although the few studies that have been published to date show some discrepancies, we feel that these recently developed and more sophisticated in vivo methods will improve our insight into the relationship between the monoamine and other transmitters during exercise. Continued quantitative and qualitative research needs to be conducted so that a further understanding of the effects of exercise on brain neurotransmission can be gained.

The health benefits of exercise include favourable physiological, psychological and biochemical changes. Research into the physiological effects of exercise is usually on the muscular or neuromuscular systems even though it is apparent that there is also an influence on the CNS, with convincing evidence that several neurotransmitters are involved in control of locomotion.[2-8]

Fatigue during prolonged exercise has traditionally been attributed to the occurrence of a 'metabolic end-point', where muscle glycogen levels are depleted, plasma glucose levels are reduced, and plasma free fatty acid levels are elevated.[9] There is also a 'central fatigue hypothesis'[10-12] which is based on the increase in the level of brain serotonin (5-hydroxytryptamine; 5-HT) during exercise. However, the physiological mechanisms for central fatigue are largely unexplored.

This review will focus on the effects of exercise on neurotransmission, especially the influence of exercise on the monoaminergic systems. We will discuss the possible role and interaction of the neurotransmitters and their precursors in central fatigue and also in motor behaviour. Finally, recent developments in the direct measurement of neurotransmitter release with microdialysis and voltammetry are presented.

This work includes both animal and human studies. Most of the studies that examined the effects of exercise on brain neurotransmitters were performed on animals. When exercise performance and precursor loading or pharmacological manipulations are discussed we also included the results of human studies.

1. Biosynthesis of Monoamines

The biogenic amines include the catecholamines, dopamine, noradrenaline (norepinephrine), adrenaline (epinephrine), and the indolamine, serotonin. Tyrosine is the common amino acid precursor of all catecholamines, while the precursor of serotonin is the essential amino acid tryptophan. Monoaminergic neurons modulate a wide range of functions in the central nervous system.[13] Noradrenergic neurons are involved in cardiovascular function, sleep and analgesic responses, while dopaminergic neurons are linked with motor function[2] and serotonergic activity is associated with pain, fatigue, appetite and sleep.[13]

1.1. The Dopaminergic System

Dopaminergic cell groups are found in the mesencephalon, the diencephalon and the telencephalon. The main ascending dopaminergic pathways include the nigrostriatal tractus, the ventral mesostriatal (or mesolimbic) pathway and the tubero-infundibular system which arises from cells located in the diencephalon.[14]

The rate-limiting step in the biosynthesis of dopamine is the hydroxylation of tyrosine to dihydroxyphenylalanine (dopa) by the enzyme tyrosine hydroxylase. The majority of tyrosine hydroxylase is located in catecholamine nerve terminals. Tyrosine hydroxylase activity can be inhibited by the catecholamines, suggesting a feedback inhibitory effect. Dopa is decarboxylated to dopamine by the enzyme dopa-decarboxylase (aromatic amino acid decarboxylase). The activity of
this enzyme is not rate-limiting in the synthesis of the catecholamines, and is therefore no regulating factor in their formation. Dopamine is in normal physiological conditions first metabolised to 3,4-dihydroxyphenylacetic acid (DOPAC) by monoamine oxidase and aldehyde oxidase. DOPAC is then further metabolised into homovanillic acid by catechol-\(O\)-methyltransferase.\textsuperscript{14}

1.2 The Noradrenergic System

The neurons that synthesise noradrenaline (norepinephrine) are restricted to the pontine and medullary segmental region. The locus coeruleus is quantitatively the most important noradrenergic nucleus in the brain. Its efferent fibres constitute a major ascending pathway, the dorsal noradrenergic
bundle. Along its course different branches emerge to innervate a large number of mesencephalic areas (dorsal raphe nucleus, thalamus, hypothalamus, hippocampus, septum and cortex).

In the noradrenergic neurons dopamine is converted into noradrenaline (norepinephrine) through dopamine β-hydroxylase. The enzymes responsible for the catabolism of noradrenaline are monoamine oxidase and catechol-O-methyltransferase. The main metabolite of noradrenaline is 3-methoxy-4-hydroxyphenylethanol (3-MHEPA) [fig. 1].

1.3 The Serotonergic System

Serotonin-containing neurons are present in the mesencephalon, pons and medulla oblongata. They are mainly located in the raphe nuclei. Efferent fibres innervate the substantia nigra, various thalamic centres, the nucleus caudatus, the putamen, the nucleus accumbens, the cortex and the hippocampus. Other serotonergic cells innervate the ventral horn of the spinal cord and the medulla.

The synthesis of serotonin requires two enzymatic steps. The dietary amino acid precursor tryptophan is first hydroxylated by a tryptophan hydroxylase to L-5-hydroxytryptophan (L-5-HTP) and then decarboxylated to serotonin. Serotonin itself is metabolised by 2 enzymes (aldehyde dehydrogenase and monoamine oxidase) to 5-hydroxyindoleacetic acid (5-HIAA) [fig. 2].

2. Exercise and Brain Monoamines

The first reports that examined the influence of exercise on brain neurotransmitters appeared in the 1960s. These studies mostly used exercise as a stress model, or compared exercise with other stressors such as exposure to cold. Since then several studies have continued to use this approach comparing or combining exercise with other stressors such as foot-shock, tail pinch, immobilisation, or restraint. Other studies described the influence of exercise on brain monoamines as a possible intervention in affective disorders and depression. Most of these animal studies, however, examined brain monoamine levels with acute and chronic exercise protocols to explore the effects of a physiological stimulus on brain neurotransmission.

2.1 The Noradrenergic System

Studies that examined whole brain noradrenaline levels after acute bouts of exercise (running or swimming) mostly found a decrease, no effect, or a small not-significant increase in

![Fig. 2. Biosynthesis and catabolism of serotonin (5-hydroxytryptamine; 5-HT).](image_url)
<table>
<thead>
<tr>
<th>Reference</th>
<th>Animals</th>
<th>Exercise</th>
<th>Training</th>
<th>Brain area</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cicardo et al.[26]</td>
<td>Wistar rats</td>
<td>Swimming in 23°C</td>
<td>To exhaustion</td>
<td>Whole brain</td>
<td>↓</td>
</tr>
<tr>
<td>Morel[19]</td>
<td>Abino mice</td>
<td>Running wheel</td>
<td>Spontaneous activity</td>
<td>Whole brain</td>
<td>Administration of αMT: NA ↓</td>
</tr>
<tr>
<td>Moore &amp; Lariviere[19]</td>
<td>S-D rats Female</td>
<td>Swimming</td>
<td>4h 23°C or 37°C</td>
<td>Whole brain</td>
<td>↓</td>
</tr>
<tr>
<td>Barchas &amp; Freedman[16]</td>
<td>S-D Male (200g)</td>
<td>Treadwheel: 3h, 1.8 m/min</td>
<td>Whole brain</td>
<td>10% ↓</td>
<td></td>
</tr>
<tr>
<td>Sheldon et al.[39]</td>
<td>Swiss-Webster mice Female</td>
<td>Swim to exhaustion</td>
<td>15°C: 15 → 30 mins</td>
<td>11% ↓</td>
<td>no change in synthesis and turnover of catecholamines (no specific determination)</td>
</tr>
<tr>
<td>Broocks et al.[40]</td>
<td>Wistar rats Male</td>
<td>Running wheel</td>
<td>Spontaneous running</td>
<td>Hypothalamus</td>
<td>↑ In both groups</td>
</tr>
<tr>
<td>Tma &amp; Hellhammer[21]</td>
<td>Water rats Male</td>
<td>Running wheel</td>
<td>Spontaneous running</td>
<td>Preoptic area</td>
<td>↑ In both groups</td>
</tr>
<tr>
<td>Stone[33]</td>
<td>S-D Male</td>
<td>Motor driven running wheel</td>
<td>3h 5.0 m/min → 8 m/min</td>
<td>NA</td>
<td>MPHG</td>
</tr>
<tr>
<td>Östman &amp; Nyback[31]</td>
<td>S-D Male</td>
<td>Swimming in 35°C</td>
<td>17 weeks, 1 → 2.5 h/day</td>
<td>Whole brain</td>
<td>↑ In all runners</td>
</tr>
<tr>
<td>Aackson et al.[12]</td>
<td>Male</td>
<td>Treadmill: 25 m/min 4° incline</td>
<td>90 mins</td>
<td>Whole brain</td>
<td>↑ In runners - control compared to control</td>
</tr>
<tr>
<td>Brown &amp; Van Haus[19]</td>
<td>S-D Male</td>
<td>Running wheel</td>
<td>8 weeks, 1 h/day</td>
<td>Whole brain</td>
<td>↑ In trained animals</td>
</tr>
<tr>
<td>Decastro &amp; Duncan[25]</td>
<td>Long-Evans hooded rats Male</td>
<td>Running wheel</td>
<td>Operant conditioning: 8 weeks, 5 days/week, 2 h/day</td>
<td>Whole brain</td>
<td>Small ↑ (NS)</td>
</tr>
</tbody>
</table>

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Table I. Contd

<table>
<thead>
<tr>
<th>Reference</th>
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<th>Training</th>
<th>Brain area</th>
<th>Results</th>
</tr>
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<tbody>
<tr>
<td>Brown et al.[31]</td>
<td>Wistar</td>
<td>Treadmill</td>
<td>8 weeks, 5 days/week, 30 m/min</td>
<td>Whole brain</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>Female n = 40</td>
<td></td>
<td>30 m/min or fat (diet)</td>
<td>Cerebellum</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Midbrain</td>
<td>↑</td>
</tr>
<tr>
<td>Brown et al.[41]</td>
<td>Female rats</td>
<td>Treadmill</td>
<td>30 mins, 6 weeks</td>
<td>Telencephalon</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td>n = 36</td>
<td></td>
<td></td>
<td>(mainly Cortex)</td>
<td>↓</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Medulla oblongata</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hypothalamus</td>
<td>↓</td>
</tr>
<tr>
<td>Elam et al.[29]</td>
<td>Wistar Kyoto</td>
<td>Running wheel; spontaneous</td>
<td>5 km/12h</td>
<td>Limbic forebrain</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>Male n = 25</td>
<td>running, animals killed</td>
<td>7 days</td>
<td>striatum, brain</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>immediately or 24 h after running</td>
<td></td>
<td>stem, cortex,</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>period</td>
<td></td>
<td>spinal cord</td>
<td>↑</td>
</tr>
<tr>
<td>Blomstrand et al.[29]</td>
<td>Wistar rats</td>
<td>Treadmill: sedentary, 17 m/min 7°</td>
<td>Sedentary accommodation: 10 m/min/day → low speed</td>
<td>Cortex</td>
<td>Not measured</td>
</tr>
<tr>
<td></td>
<td>Female (200–220 g)</td>
<td>incline RTE: trained 30–35</td>
<td>Trained: 11 weeks 6 days/week 1 h/day, 3 cm/min 7° incline</td>
<td>Cerebellum</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>n = 15/group</td>
<td>m/min 7° incline</td>
<td></td>
<td>Hippocampus</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Striatum</td>
<td>67% ↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Brainstem</td>
<td>50% ↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hypothalamus</td>
<td>32% ↑ (NS)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hypothalamus</td>
<td>15% ↓ (NS)</td>
</tr>
<tr>
<td>Sudo[27]</td>
<td>S-D</td>
<td>Swimming 4 h; 35°C water</td>
<td></td>
<td>Hypothalamus</td>
<td>↑</td>
</tr>
<tr>
<td>Gordon et al.[17]</td>
<td>S-D</td>
<td>Rotating drum; 7 rpm (high speed)</td>
<td>3 and 5 h (1 exc group with oMT)</td>
<td>Hypothalamus</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>Female (160–220 g)</td>
<td>7 rpm (high speed)</td>
<td>1 h + tyrosine C14</td>
<td>Striatum</td>
<td>↓ With exc (slight depletion)</td>
</tr>
<tr>
<td></td>
<td>n = 40 (TTL)</td>
<td></td>
<td></td>
<td>Brain stem</td>
<td>↓ With oMT + exc</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↑ Radioactivity with exc</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↑ Symmetry at NA</td>
</tr>
<tr>
<td>Hoyes et al.[29]</td>
<td>S-D</td>
<td>Treadmill; RTE or after (6, 11,</td>
<td>Striatum</td>
<td>Not measured</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male (350–400 g)</td>
<td>36 m/min and 16.5 mins</td>
<td></td>
<td>Brainstem</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>n = 25 (± 5/group)</td>
<td></td>
<td></td>
<td>Hypothalamus</td>
<td>↓ Progressively during exc</td>
</tr>
<tr>
<td>Lukaszyn et al.[39]</td>
<td>Wistar</td>
<td>Treadmill: 20 mins</td>
<td>Cortex, striatum</td>
<td>Hypothalamus</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>Male (150–220 g)</td>
<td>30 m/min</td>
<td>20 mins</td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td>n = 5/group</td>
<td></td>
<td></td>
<td>Midbrain, cortex</td>
<td>↑</td>
</tr>
</tbody>
</table>

Abbreviations: oMT = O-methyl-p-tyrosine; exc = exercise; h = hour(s); MHPG = 3-methoxy-4-hydroxyphenylglycol; NA = noradrenaline; NS = not significant; RTE = run to exhaustion; S-D = Sprague-Dawley rats; sig. = significant; ↑ indicates no significant change; ↓ = increase; ↓ = decrease; - = no significant difference.

Brain noradrenaline levels,[12,25] (see Table I)

Whole brain noradrenaline levels increased after chronic exercise training.[12,25,30,32] Some studies examined noradrenaline levels in specific brain regions. Noradrenaline levels decreased due to acute exercise in brain stem,[17,33,35] hippocampus,[56] pons-medulla,[31] midbrain,[37] and hypothalamus,[33,35,37,38] while noradrenaline levels in the striatum,[36,39] cortex,[48] and preoptic area[40] increased. Interestingly, hypothalamic noradrenaline levels have been shown to increase in food-restricted rats.[56] The same study measured MHPG and found an increase in most brain regions indicating an activation of noradrenaline catabolism.

Studies that examined the effects of exercise training on noradrenaline levels in different brain regions found mostly an increase or no significant result.[30,31,39,41] Stone[33] examined alterations in storage of [³H]noradrenaline in hypothalamus of
rats during 3 hours of exercise on a motor driven running wheel. They further compared exercise with injections of reserpine (inhibition noradrenaline storage) or α-methyl-p-tyrosine (inhibition noradrenaline synthesis). Running did not alter storage of noradrenaline, therefore the authors concluded that it was likely that the noradrenaline depletion during running was derived from newly synthesised noradrenaline and not from reuptake mechanisms.\[33\] One study\[23\] investigated the accumulation of dopa as an index of tyrosine hydroxylation activity in order to obtain an indication of the monoamine synthesis rate. It found higher dopa levels in the brain stem indicating an increased synthesis of noradrenaline in this predominantly noradrenaline-rich region.\[21\]

Only one study reported changes in adrenaline level following 4 hours of swimming.\[37\] The adrenaline levels in hypothalamus, pons-medulla and midbrain showed a gradually decrease, with a significant decrease in the second part of the exercise period.\[37\]

It seems that acute exercise results in a depletion of brain noradrenaline probably because of an acceleration in noradrenaline turnover by activating tyrosine hydroxylase activity.\[13\] while chronic exercise has been found to elevate brain noradrenaline levels. These adaptations are region specific.

2.2 The Dopaminergic System

Dopaminergic neurons are considered to be critical components in the motor system.\[2\] A number of studies have examined the effect of acute or chronic exercise on dopamine synthesis and metabolism (table II). Two studies\[17,29\] used the incorporation of $[^{14}C]$tyrosine into $[^{14}C]$dopamine and did not find changes in central dopamine synthesis and turnover. Another early study\[21\] however, found an increase in homovanillic acid level in mice following swimming and running. Chaoulloff et al.\[42\] also confirmed an increased dopamine metabolism in the whole brain of rats from running.

A number of studies used trained animals to study the effects of chronic exercise or the effects of an acute exercise session following training on brain dopamine levels. Whole brain dopamine level was increased in rats killed 48 hours after an 8-week training period.\[25\] A 1-week training model was used to examine brain dopamine metabolism and found that the sum of the levels of DOPAC and homovanillic acid was increased with running and remained elevated throughout the first hour of recovery.\[43\] Two studies\[12,30\] did not find a significant influence of exercise on whole brain dopamine level of trained rats.

Most studies examined the effects of acute and chronic exercise on regional dopaminergic systems. One study\[38\] examined the effects of 20 minutes of exercise on regional dopamine levels in untrained rats and found decreased levels in all brain regions examined. Swimming for 4 hours in 35°C water resulted in a small but not significant decrease in dopamine level in striatum and midbrain.\[35\] Other studies found increased dopamine, DOPAC and homovanillic acid in striatum,\[20,35\] brainstem\[35\] and hypothalamus.\[39\]

In the same region (the hypothalamus) the level of dopamine and its metabolites was unchanged although the ratio DOPAC + homovanillic acid to dopamine increased.\[22\] These results indicate an increased dopamine synthesis and metabolism, although in another study treatment with α-methyl-p-tyrosine had no effect on dopamine synthesis in brainstem, and it decreased DOPAC level in the whole brain in control and exercising rats.\[43\]

The fact that dopamine metabolism in rat striatum is involved in movement has been shown.\[2\] The data showed that there is a very close relationship between dopamine production and all aspects of motor behaviour (speed, direction and body posture). The dopamine level in the nucleus accumens appears to be a marker for the speed of animals while dopamine level in the caudate is more related to posture.\[2\]

The effect of training on regional dopamine level was examined in several studies. In some studies the animals ran, while others examined regional transmitter levels without an acute training session. The level of dopamine and its metabolites was found to increase in hypothalamus,\[9,41,43\] mid-
<table>
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<th>Brain area</th>
<th>Results</th>
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<tbody>
<tr>
<td>Sheldon et al. [29]</td>
<td>Swiss-Webster male, Female (25-30g)</td>
<td>Treadmill: 5.4 mi/min, 150 mins + tyramine C&lt;sup&gt;14&lt;/sup&gt;</td>
<td>Whole brain</td>
<td>No change in synthesis and turnover of catecholamines</td>
<td></td>
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<tr>
<td>Bliss &amp; Alford [31]</td>
<td>Male, Female ± 35g</td>
<td>Swimming: 1h 37°C, Spontaneously running: 30 mins</td>
<td>Whole brain</td>
<td>Swimming: ↑HVA, ↑DA metabolism</td>
<td></td>
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<tr>
<td>Freed &amp; Yamamoto [32]</td>
<td>S/D Male (250-350g), n = 6/group</td>
<td>Treadmill: 20 mins, 1.42 × 7.2 mm/min</td>
<td>Sphincter, n. accumbens</td>
<td>↑DA &amp; DOPAC (posture &amp; direction)</td>
<td></td>
</tr>
<tr>
<td>Speciale et al. [33]</td>
<td>S/D Male (250-300g), n = 6/group</td>
<td>Running wheel: 6.3 mm/min, 11: 12.6 mm/min</td>
<td>Striatum, n. accumbens, prefrontal cortex</td>
<td>↑DA metabolism in striatum not frontal cortex</td>
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<tr>
<td>Gordon et al. [34]</td>
<td>Female (160-220g), n = 46 (TTL)</td>
<td>Rotating drum, 7 rpm (high speed), 3 and 5h (1 sec group with uMT), 1h + tyramine C&lt;sup&gt;14&lt;/sup&gt;</td>
<td>Brain stem</td>
<td>↑DA metabolism in striatum not frontal cortex</td>
<td></td>
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<td>Heyda et al. [35]</td>
<td>S/D Female (280-400g), n = 25 (SG)</td>
<td>Treadmill: 36.0 mi/min, RETE or after 6, 11, 16.5 mins</td>
<td>Sphincter</td>
<td>No significant effect on DA synthesis</td>
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<td>Lukaszyk et al. [36]</td>
<td>Wistar rats, Male (150-220g), n = 5/group</td>
<td>Treadmill: 20 mins, 5 weeks, 5 days a/w, 60 mins/day incline 1° to 4°</td>
<td>Posterior hypothalamus</td>
<td>DA, ↓DOPAC, ↓HVA, ↓DOPAC + HVA, ↓DA</td>
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<tr>
<td>Acorworth et al. [37]</td>
<td>Male rats</td>
<td>Treadmill: 20 mins, 25 mm/min, 4° incline</td>
<td>Cortex, hippocampus, hypothalamus, striatum, midbrain, cerebellum</td>
<td>DA, ↓DOPAC, ↓HVA, ↓DOPAC + HVA, ↓DA</td>
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<td>Chrousouff et al. [38]</td>
<td>Wistar rats, Male (250-300g), n = 5 (brain), n = 6 (CSF)</td>
<td>Treadmill: 20 mins, 20 mm/min, 1 week, 4 to 5 times, last session 1h at 20 mm/min</td>
<td>Whole brain</td>
<td>DA, ↓DOPAC, ↓HVA, ↓DOPAC + HVA, ↓DA</td>
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<tr>
<td>Chrousouff et al. [39]</td>
<td>Wistar rats, Male (250-300g), n = 5/group</td>
<td>Treadmill: 20 mins, 20 mm/min, 1 week, 4 to 5 times, last session 1h at 20 mm/min</td>
<td>Whole brain</td>
<td>DA, ↓DOPAC, ↓HVA, ↓DOPAC + HVA, ↓DA</td>
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<td>Bailey et al. [40]</td>
<td>Wistar rats, Female</td>
<td>Treadmill accommodation: 3.4 weeks, speed × var time, last session 20 mm/min, 5%, 30 mins</td>
<td>Hippocampus, Striatum, Midsleep</td>
<td>Exec + mCPP, DA, ↓DOPAC, ↑DOPAC, ↑DOPAC + HVA</td>
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<th>Reference</th>
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<th>Brain area</th>
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</thead>
<tbody>
<tr>
<td>Bloemendal et al.</td>
<td>Wistar rats Female (200-250g) n = 5/group</td>
<td>Treadmill: sedentary, ±17 mm/7° incline RTE; trained, ±30-35 mm, ±10° incline</td>
<td>Sedentary accommodation 10 mins/day at slow speed Trained: 1 week 6 days/week, 1 hr/day</td>
<td>Cortex Cerebellum Hippocampus Striatum Brainstem Hypothalamus</td>
<td>Sedentary Not measured Not measured Below detect limit Below detect limit 27% ↑ NS 67% ↑ NS 67% ↑ NS Intrav, post run 24th postrun</td>
</tr>
<tr>
<td>Elam et al.</td>
<td>Wistar Kyoto Male n = 25</td>
<td>Running wheel: spontaneous running animals killed immediately or 24th after last running period 5 km/12h 7 days</td>
<td>Limbic forebrain (incl. n. accumbens) Striatum Brainstem, cortex, spinal cord</td>
<td>DopA ↓</td>
<td></td>
</tr>
<tr>
<td>Hoffmann et al.</td>
<td>Spontaneously hypertensive rats Male (200-300g) n = 32 (8/group)</td>
<td>Running wheel: spontaneous running, animals killed 1 hr; or 47-48th after last run period 5 week period; 4 km/24hr TTL; 7 weeks</td>
<td>Limbic forebrain Cortex, striatum, diencephalon, brainstem</td>
<td>DopA ↑</td>
<td></td>
</tr>
<tr>
<td>Brown &amp; Van Hout</td>
<td>S-D Male n = 80</td>
<td>Running wheel 60 min 8 weeks, 1 hr/week</td>
<td>Whole brain</td>
<td>No effect in either period</td>
<td></td>
</tr>
<tr>
<td>Brown et al.</td>
<td>Female rats n = 38</td>
<td>Treadmill 30 mins 8 weeks, 1 hr/week</td>
<td>Telencephalon (mainly cortex) Mesencephalon, obex, medulla oblongata, hypothalumus</td>
<td>DA ↑</td>
<td></td>
</tr>
<tr>
<td>Decastro &amp; Duncan</td>
<td>Long-Evans hooded rats Male (296-375g) n = 12 (6 pairs)</td>
<td>Running wheel: Animals killed 48 hrs after final training</td>
<td>Operant conditioning technique 8 weeks, 5 days/week, 24hr/day (ran ± 1.2 km/day) (not conditioned animals ran ± 0.5 km/day)</td>
<td>Whole brain</td>
<td>DA ↑</td>
</tr>
<tr>
<td>Gillam et al.</td>
<td>S-D Male (330-550g) n = 30</td>
<td>Treadmill: animals killed 48 hrs after final training</td>
<td>Endurance: 27 min/min, 12 weeks, 6 days/week or interval training</td>
<td>Striatum</td>
<td>Endurance &amp; interval group were not exp. different from each other DA ↑ DA ↑</td>
</tr>
<tr>
<td>MacRae et al.</td>
<td>S-D Male young adults</td>
<td>Treadmill</td>
<td>6 months, 6 days/week, 27 min/min</td>
<td>Striatum</td>
<td>No affect on steady-state levels of DA or metabolites DA binding sig. ↑ in runners</td>
</tr>
<tr>
<td>Bailey et al.</td>
<td>Wistar rats Male (300-400g) TTL = 72 (n = 8/group)</td>
<td>Treadmill: 60 mins or exhaustion, 20 min/min, 5% incline Treadmill acclimation, 3-4 weeks, speed = run time/10, last session: 20 min/min, 5%, 30 mins</td>
<td>Midbrain Striatum Hypothalamus Hippocampus</td>
<td>DA ++ Dopac ++ Dopac = Dopac =</td>
<td></td>
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</table>
Exercise and Brain Neurotransmission

brain\textsuperscript{[9,43,44]} prefrontal cortex,\textsuperscript{[43]} hippocampus\textsuperscript{[43,44]} and striatum.\textsuperscript{[9,41,43]} Bailey et al.\textsuperscript{[9]} also found elevated dopamine and DOPAC levels after 1 hour of exercise, however, these increases were not as great as reported. The hippocampal dopamine levels did not change as a result of exercise.\textsuperscript{[9]}

Another group\textsuperscript{[23]} used spontaneous, long term running in a wheel cage. Exercising animals were killed 1 to 2 hours or 24 hours after the last running period. By giving the animals NSD (an aromatic amino acid decarboxylase inhibitor) 30 minutes before killing the authors measured the accumulation of dopa, an index for monoamine synthesis rate in different brain regions. The results showed a decreased rate of dopamine synthesis in dopamine-rich brain regions, while dopa levels were considerably higher in noradrenaline regions (brainstem) indicating an increased synthesis of noradrenaline in this region. All these alterations were normalised after 24 hours. However, measurement of dopa accumulation does not enable an evaluation of the in vivo physiological activity of the monoamine pathways.\textsuperscript{[23]}

Three studies\textsuperscript{[24,45,60]} used \textsuperscript{[3}H\textsuperscript{]}-piperone receptor binding to get an indication of transmitter dynamics. An operant conditioning model with positive reinforcement has been used to induce exercise.\textsuperscript{[25]} Although dopamine levels increased, they found \textsuperscript{[3}H\textsuperscript{]}-piperone binding to be decreased in whole brain homogenates. The authors state that caution must be exercised in interpreting these results as a change solely in dopamine receptor binding. A more specific regional determination could result in different findings. This was confirmed by Gilliam et al.\textsuperscript{[45]} who showed that animals, exercised on a moderate to high intensity endurance or interval running protocol, showed significantly higher \textsuperscript{[3}H\textsuperscript{]}-piperone receptor binding than sedentary controls.

The effects of 6 months of endurance training on the relationships among steady-state in vivo levels of dopamine and its metabolites and the affinity and density of striatal-D\textsubscript{2} receptors were determined.\textsuperscript{[46]} Endurance training had no effect on steady-state levels of dopamine or its major meta-
bolites in striatum. The major finding of this study was that 6 months of endurance training alters neurochemical markers in the nigrostriatal dopamine system in young adult rats. Binding of [3H]-spiiperone to D2 receptors and the ratio of binding to levels of DOPAC were enhanced in the runners. Together these results suggest that a shift in dopamine function may occur as a result of exercise, either due to altered dopamine release or to changes in D2 binding sites. However, as has already been indicated, spiperone will also bind to other (serotonin) receptor types. It is difficult to draw conclusions from these studies because there is no uniformity with the study methods used. Neurotransmitter levels in whole brain or brain regions are just an indication of the amount neurotransmitter, and give us no information concerning neuronal activity. Receptor binding studies used are not specific to one receptor type.

The dopaminergic nerve terminals appear to play an important role in the regulation of locomotor activity and it seems that the influence of acute or chronic exercise is region specific. However, it should be recognised that the so called ‘motor circuit’ containing neurons from the striatum, substantia nigra, cerebral cortex and thalamus, interact constantly through several transmitters and receptor types. It is difficult, if not impossible, to register this dynamic and constant interaction with brain homogenate preparations.

2.3 The Serotonergic System

A number of studies have examined brain serotonin and 5-HIAA levels with acute and chronic exercise (table III). Chaouloff and his co-workers have published several papers on this topic. [15,24,43,49-52] Except for one study [28] that found no change in serotonin level, whole brain serotonin and 5-HIAA increase following an acute bout of exercise. [12,16,53] However, in trained rats it seems that brain serotonin level is unaltered while 5-HIAA level increases. The first studies by Chalouff et al. [42,49] showed that exercise increased brain and cerebrospinal fluid tryptophan and 5-HIAA, indicating an increase in serotonin synthesis and metabolism. The same authors did not find a difference in basal brain serotonin levels between short and long term trained rats. A single running session did not change serotonin level in the rats trained for 1 week, but serotonin was diminished in rats trained for 8 weeks, probably indicating a different serotonin utilisation. [51]

Acute and chronic exercise studies have found both increased and decreased levels and turnover of serotonin and 5-HIAA, depending on the brain region of interest. Striatal, hippocampal and midbrain serotonin and 5-HIAA levels increased after a training session, or after an acute bout of exercise in trained rats. [9,31,39,44,52,54]

Dey et al. [26] studied serotonin and 5-HIAA alterations in different brain regions following acute (1 hour swim) and chronic exercise (4 weeks of swimming, 6 days/week). Acute exercise significantly increased the synthesis and metabolism of serotonin in the brain stem and hypothalamus, and there were no changes in cerebral cortex and hippocampus. Chronic exercise activated not only the synthesis but also the metabolism of serotonin in cerebral cortex. One week after the termination of training this neuronal adaptation was still present. In brain stem, serotonin turnover increased immediately after the training session. In hippocampus a delayed effect was observed, because serotonin level was unaltered immediately after the training, but its turnover decreased after 1 week of rest. In hypothalamus serotonin and 5-HIAA decreased immediately after training, followed by a rebound increase in their levels after 1 week of post-training rest. [26]

Two studies [23,24] used a spontaneous wheel running model to avoid other stressors such as footshock to examine changes in serotonin metabolism and turnover. An aromatic amino acid decarboxylase (AADC) inhibitor was used to measure 5-HTP (the direct precursor of serotonin) accumulation, which can give an estimate of serotonin synthesis. [23] There were no statistically significant differences in 5-HTP. Chatouff et al. [34] however, found regional differences in tryptophan...
<table>
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<tr>
<th>Reference</th>
<th>Subjects</th>
<th>Exercise</th>
<th>Training</th>
<th>Brain area</th>
<th>Results</th>
</tr>
</thead>
</table>
| Batchas & Freedman* | S-D Male (200g) n = 50 | Treadmill: 3h, 1.8 min/min | Whole train | T
| Speciale et al. | S-D Male (250-300g) n = 6 | Running wheel: 6.3 m/min, 1h | Striatum (n. caudatus) | No difference serotonin or 5-HIAA |
| Heyes et al. | S-D Male (350-400g) n = 25 (5/group) | Treadmill: 30 m/min, RTE or after: 6, 11, 16.5 mins | Striatum, brainstem, hypothalamus | Serotonin unchanged in all regions |
| Lukaszuk et al. | Water rats Male (150-220g) n = 5/group | Treadmill: 20 mins, 30 m/min | Cortex, hippocampus, hypothalamus, striatum, midbrain, cerebellum | Serotonin in all regions but only statistically sig. in striatum cortex, midbrain |
| Acworth et al. | Male rats n = 23 (TTL, 5/group) | Treadmill: 90 mins, 25 m/min, 4 indlin | Whole train | Serotonin, 5-HIAA |
| Chasloff et al. | Water rats Male (220g) | Treadmill: 60 mins, 120 mins | Whole train | Serotonin, but serotonin metabolism |
| Chasloff et al. | Water rats Male (250-300g) n = 5 (CSF) | Treadmill: 60 mins, 20 m/min | Whole train | 5-HIAA indicating serotonin turnover return to normal after 1h |
| Chasloff et al. | Water rats Male (250-300g) n = 4 (CSF) | Treadmill: 120 mins, 20 m/min | Whole train | 5-HIAA |
| Acworth et al. | Male rats | Treadmill: 90 mins, 25 m/min, 4 indlin | Whole train | Serotonin, 5-HIAA sign |
| Chasloff et al. | Water rats Male (200-250g) n = 5/group | Treadmill: 60 mins, 30 m/min | Whole train | Serotonin, 5-HIAA |
| Bailey et al. | Female n = 5 | Treadmill: 50 mins 30 min/min 5% | Midbrain | Serotonin, 5-HIAA |

Exercise and Brain Neuronal Transmission

Source: Mc. 2013, 105
<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects</th>
<th>Exercise</th>
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<th>Brain area</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hoffman et al.</td>
<td>Spontaneously hypertensive rats</td>
<td>Running wheel</td>
<td>5 week period</td>
<td>Limbic forebrain, Brainstem, Striatum, cortex, diencephalon</td>
<td>Serotonin &amp; 5-HIAA ↑ sign. (1 to 2 h)↑ - serotonin trace ↑ 5-HIAA ↓ sign, 48h postinfusion NS serotonin or 5-HIAA</td>
</tr>
<tr>
<td>Brown et al.</td>
<td>Wistar rats</td>
<td>Treadmill &amp; 30 min/min</td>
<td>8 weeks, 5 days/week, 30 min/day (normal diet or fat diet)</td>
<td>White brain, Cerebellum, Medulla</td>
<td>Serotonin ↑</td>
</tr>
<tr>
<td>Helminn et al.</td>
<td>Wistar rats</td>
<td>Running wheel</td>
<td>Spontaneous running</td>
<td>Porvomedia, Thalamus, Hypothalamus, Medulla, Septum, Hippocampus, Striatum</td>
<td>Serotonin ↑, 5-HIAA ↓</td>
</tr>
<tr>
<td>Cicardi et al.</td>
<td>Wistar rats</td>
<td>Swimming in 23°C to exhaustion</td>
<td></td>
<td>White brain</td>
<td>Serotonin ↑</td>
</tr>
<tr>
<td>Dey et al.</td>
<td>Albino rats</td>
<td>Swimming, 60 mins, (35-36°C)</td>
<td>4 weeks, 5 days/week, 30 min/day, Spontaneous 3 to 7 days post exp.</td>
<td>Cortex, Hippocampus, Hypothalamus, Brainstem</td>
<td>Acute: Serotonin ↑, 5-HIAA ↑ NS Serotonin ↑, NS, 5-HIAA ↑ - Serotonin ↑, 5-HIAA ↑ Serotonin ↑, 5-HIAA ↑</td>
</tr>
<tr>
<td>Wilson et al.</td>
<td>Male hooded rats</td>
<td>Treadmill</td>
<td>4 weeks accommodation speed &amp; run time gradually increase</td>
<td>Hippocampus, Fornix, Cortex, Hypothalamus, Brainstem</td>
<td>Serotonin ↑ (NS) Serotonin ↑ (NS)</td>
</tr>
<tr>
<td>Belley et al.</td>
<td>Wistar rats</td>
<td>Treadmill</td>
<td>Teadmill: accommodation 3-4 weeks; speed = run time 1st test session 20 min/day, 5%, 30 min</td>
<td>Medulla, Striatum, Hypothalamus, Hippocampus</td>
<td>Serotonin ↑, 5-HIAA ↑ Serotonin ↑, 5-HIAA ↑ Serotonin ↑, 5-HIAA ↑ Serotonin ↑, 5-HIAA ↑</td>
</tr>
<tr>
<td>Chaouloff et al.</td>
<td>Wistar rats</td>
<td>Treadmill</td>
<td>Teadmill accommodation: 1 week-6 days/week last session 1 h, 20 min</td>
<td>Medulla, Striatum, Hippocampus</td>
<td>5-HTP accum. ↑ Serotonin ↑ 5-HIAA ↑ Serotonin ↑ 5-HIAA ↑</td>
</tr>
<tr>
<td>Study</td>
<td>Rats</td>
<td>Condition</td>
<td>Treatment</td>
<td>Brain Region</td>
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<tr>
<td>Romanov and Gabrielski (2020)</td>
<td>Male 10–100g</td>
<td>Treadmill 90 mins.</td>
<td>74 mins + 10% control</td>
<td>Whole brain</td>
<td>T &gt; 17% serotonin</td>
</tr>
<tr>
<td>Berglund et al. (2019)</td>
<td>Male 200–220g</td>
<td>Sedentary</td>
<td>2 weeks, 30 mins + 10% control</td>
<td>Cortex, cerebellum</td>
<td>No difference (control)</td>
</tr>
<tr>
<td></td>
<td>n = 5 group</td>
<td>Slow speed</td>
<td>2 days + 10% control</td>
<td>Hippocampus</td>
<td>No difference (control)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8 days/week, 1 hr</td>
<td>10 mins/day, 10% control</td>
<td>Brainstem</td>
<td>No difference (control)</td>
</tr>
<tr>
<td></td>
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<td>After last running</td>
<td>10 mins/day, 10% control</td>
<td>Hypothalamus</td>
<td>No difference (control)</td>
</tr>
<tr>
<td>Elrod et al. (2018)</td>
<td>Male 25</td>
<td>Running wheel</td>
<td>7 days, spontaneous running</td>
<td>Limbic forebrain,</td>
<td>No effect on 5-HTP accum.</td>
</tr>
<tr>
<td></td>
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<td>6 km/2 h</td>
<td>striatum, cortex,</td>
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<td>spinal cord</td>
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</table>

*Note: In food restricted runners compared with control.
* Comparing to not food restricted runners.
* Symbols and abbreviations: acm = accumulation; 5HTP = 5-methyltryptamine; AMPH = amphetamine; C = control; exc = exercise; EXH = exhaustion; h = hour(s); liv = livid; mCPP = 5-HT2 receptor agonist; NS = not significant; RTE = run to exhaustion; serotonin = 5-hydroxytryptamine (5-HT); 5-HIAA = 5-hydroxyindoleacetic acid; 5-HTP = 5-hydroxytryptophan; ↑ = increase; ↓ = decrease; * = indicates a change.*
brain neurotransmitter levels or have not assessed the same brain regions. The first studies used fluorescence spectrometry to determine transmitter levels, while later studies used more sensitive methods such as high performance liquid chromatography (HPLC) which allow measurement of multiple neurotransmitters and metabolites within the same sample. The discrepancies in measuring methods or statistical analysis prevents direct comparisons of results. For example, in the study of Blomstrand et al., dopamine level increased 65% in brainstem after exercise in trained rats. However, due to the large inter-individual variability of the responses, the authors could not reach statistical significance.

Most studies used forced locomotion on a treadmill, running wheel, or swimming. Many studies used other stressors in addition to running or swimming. Swimming in water of different temperatures or the fear of drowning, or foot-shock during running could influence results. Animals that were tested after a single exercise session ran at different speeds. The chronic exercise protocols mostly used an accommodation period in which running speed and run time gradually increased, but these protocols varied from study to study. Only a few studies examined the effects of their training programme on endurance capacity or fitness level of the animals.

Measuring neurotransmitter levels in homogenates makes no distinction between extracellular and intracellular levels, and gives no indication of neurotransmitter release. Finally, measurement of single neurotransmitter levels does not provide much information on the relationships between neurotransmitters.

2.4 Neurotransmitter Interactions

Although it is difficult to compare the above mentioned studies, it seems that physical exercise influences the synthesis and metabolism of monoamines in various brain regions. There is evidence from recent microdialysis studies that there is a reciprocal influence of various neurotransmitters in regulating their release. The various studies that examined the influence of exercise on brain neurotransmitters indicate that both central dopaminergic and serotonergic activity are influenced by exercise. Chaouloff et al. examined whether compounds known to affect dopamine activity in brain could modify the seroton response in brain during exercise. The dopamine metabolism was increased in serotonin-rich regions. Administration of amphetamine, while increasing levels of tryptophan in brain, diminished the formation of 5-HIAA. The relative inhibition of synthesis of serotonin induced by running, was thus potentiated by administration of amphetamine while α-methyl-p-tyrosine (inhibitor of catecholamine synthesis) prevented this effect of exercise, and haloperidol (dopamine antagonist) did not produce any significant change.

This possible interaction between brain serotonin and dopamine during exercise, was also proposed by Bailey et al., who examined the effects of increased serotonin activity on endurance performance and brain dopamine and serotonin turnover. They used several serotonin agonists and antagonists to examine run time to exhaustion and brain serotonin and dopamine levels. They found increased dopamine and DOPAC levels with running. At exhaustion, however, the dopamine and DOPAC levels were consistently lower than after 1 hour of exercise. Increased dopamine and DOPAC levels were significantly attenuated by m-chlorophenylpiperazine administration (mCPP is a 5-HT₁C agonist), indicating a possible impaired brain dopamine synthesis. When a general serotonin agonist (quipazine dimaleate) was administered, brain dopamine level significantly decreased at exhaustion in midbrain, and slightly decreased in striatum, hypothalamus and hippocampus. DOPAC significantly increased at exhaustion in hypothalamus and remained unchanged during exercise in the other brain regions. Administration of LY235912 (2- H1₁C/2- H1₂ antagonist) increased dopamine and DOPAC level during exercise in mid-
brain, striatum and hypothalamus, while it was un-
changed in hippocampus.

It is possible that the interaction between brain
serotonin and dopamine during prolonged exercise
could play a regulative role in the onset of fa-
tigue.\textsuperscript{[9]} However, it seems that this interaction
could be region specific and that neuroendocrine
factors also play an important role as several au-
thors already demonstrated.\textsuperscript{[9,13,64]}

Future studies need to examine the region spe-
cific interactions between multiple neurotransmit-
ters (including excitatory and inhibitory amino
acid transmitters) during exercise, and the impor-
tance of various receptors, since single neurotrans-
mitters can have both inhibitory and excitatory ef-
fects.\textsuperscript{[13]}

3. Neurotransmission and Exercise
Performance

3.1 Tryptophan, Branched Chain Amino
Acids and Neurotransmission

The variability of neurotransmitter release is reg-
ulated by a number of processes.\textsuperscript{[63]} One of
these presynaptic processes which modulates
neurotransmission is the change in neurotransmit-
ter synthesis resulting from the metabolic conse-
quences of eating or exercise.\textsuperscript{[65]} The biosynthesis
of serotonin is tightly controlled by the activity of
its rate-limiting enzymc tryptophan hydroxylase,
so increases or decreases in its substrate, trypto-
phan, trigger increases or decreases in serotonin
synthesis and metabolism.\textsuperscript{[64]} Tryptophan and
the large neutral amino acids, including the branched
chain amino acids (BCAAs) [valine, leucine, isole-
ucine] use the same carrier to enter the brain, and
therefore are competitors for transport over the
blood brain barrier. The blood level of free trypto-
phan or the ratio of free tryptophan to other large
neutral amino acids is an important parameter for
this competition.\textsuperscript{[66-68]}

Levels of circulating total and free tryptophan
in plasma, and the ratio of free tryptophan to other
large neutral amino acids, depends on several fac-
tors, e.g. the rate of lipolysis, the activity of hepatic
tryptophan pyrrolase, the uptake into the periph-
eral and central tissues.\textsuperscript{[64]} As free fatty acid levels
increase during endurance exercise, the amount of
tryptophan bound to albumin is reduced, increas-
ing the level of free tryptophan in the blood. Other
factors such as a high carbohydrate meal,\textsuperscript{[67]} insu-
lin administration,\textsuperscript{[68]} administration of L-trypto-
phan,\textsuperscript{[69]} or a combination of these factors will in-
crease the level of free tryptophan in plasma.\textsuperscript{[70]}

Since brain serotonin synthesis depends on the
plasma level of tryptophan, treatments that elevate
plasma tryptophan will promote accelerated sero-
tonin synthesis and/or metabolism.\textsuperscript{[15]} Changes in
neurotransmission caused by eating and by exer-
cise can thus affect all of the behavioural and phys-
iological functions that precursor-dependent neu-
rons happen to subserve.\textsuperscript{[65]} Table IV summarises
the studies that used precursor loading and/or phar-
macological manipulation to influence exercise
performance in human or animal models.

Serotonin has been shown to induce sleep, de-
press motor neuron excitability, influence auton-
omic and endocrine function and suppress appe-
tic.\textsuperscript{[10-12]} This led several authors to propose the
'central fatigue hypothesis'.\textsuperscript{[10-12]} In order to ex-
amine this hypothesis Blomstrand et al.\textsuperscript{[11,72,73]}
performed several studies. First, the changes in
plasma levels of amino acids were examined dur-
ing a marathon run and an army training pro-
gramme.\textsuperscript{[11]} Both types of exercise caused a signif-
ificant decrease in plasma level of BCAAs. The
plasma level of free tryptophan was found to in-
crease significantly during the race.

In two studies the effects of administration of
BCAAs on mental performance were examined.\textsuperscript{[72,73]} In one study mental performance in 6 female soccer players was studied.\textsuperscript{[73]} The
study participants were given carbohydrate drinks
with or without BCAAs. Plasma BCAAs were sig-
nificantly decreased and plasma free tryptophan
was significantly increased with the placebo car-
bohydrate drink. When ingesting the carbohydrate
plus BCAA drink, amino acid levels were signifi-
cantly increased and plasma free tryptophan was
not significantly elevated. Mental performance
<table>
<thead>
<tr>
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<th>Subject</th>
<th>Manipulation</th>
<th>Exercise</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jacobs &amp; Eubanks</td>
<td>S-D</td>
<td>Male (700g)</td>
<td>Serotonin uptake inhibitor (flupentixol)</td>
<td>Crossing 5-hydroxytryptamine (5-HTP) in vivo, activity measured during 3h</td>
</tr>
<tr>
<td></td>
<td>n = 64</td>
<td>Male (n = 6)</td>
<td>Serotonin uptake inhibitor (paroxetine)</td>
<td>Cycling to exhaustion: 70% VO₂max</td>
</tr>
<tr>
<td>Davis et al.</td>
<td>Humans</td>
<td>Male (n = 7)</td>
<td>Water: glucose or BCAA</td>
<td>Tramadol run to exhaustion: 16 m/min 5% incline</td>
</tr>
<tr>
<td>Wilson et al.</td>
<td>Humans</td>
<td>Male (n = 7)</td>
<td>mCPP</td>
<td>Tramadol to exhaustion: 20 m/min 5% incline</td>
</tr>
<tr>
<td>Veiger et al.</td>
<td>Water rats (n = 360)</td>
<td>n = 34</td>
<td>QD</td>
<td>Tramadol to exhaustion: 20 m/min 5% incline</td>
</tr>
<tr>
<td>Bailey et al.</td>
<td>Water rats</td>
<td>Male &amp; female</td>
<td>QD</td>
<td>QD: RTE ↑ in a dose-response manner</td>
</tr>
<tr>
<td>Hayes et al.</td>
<td>S-D</td>
<td>Male (300-440g)</td>
<td>6-OHDA injection</td>
<td>Tramadol: 36 min, 6-OHDA unlesioned rats</td>
</tr>
<tr>
<td>Segura &amp; Ventura</td>
<td>Humans</td>
<td>L-TRP or placebo</td>
<td>Tramadol: (80% VO₂max)</td>
<td>Tramadol: (all out 100% VO₂max)</td>
</tr>
<tr>
<td>Stonesrud et al.</td>
<td>Humans</td>
<td>L-TRP or placebo</td>
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Activity ↓ in a dose dependent manner
Serotonin more potent inhibitor than 5-HTP
Behavioral changes following 5-HTP inj, may be attributable to peripheral effects of serotonin
time to exhaustion ↓ with flupentixol
time to exhaustion ↓ with paroxetine
time to exhaustion ↓ in BCAA group compared to glucose-group
RTE ↑ in a dose response manner by mCPP administration
QD: RTE ↑ in a dose-response manner
LY 53857: RTE only ↑ in the highest dose
6-OHDA ↓ decreased RTE
Apomorphine: increased RTE
Apomorphine in 6-OHDA rats: ↑ RTE
Clonidine: no effect on RTE
† Running performance
No influence
Running wheel activity ↓ with 5-HT₆ antagonists. This effect was abolished by antagonists with high 5-HT₆ affinity
Free TRP/5HT 6/8: Mental performance ↑
Free TRP/5HT 6/8: Mental performance =
Mental performance ↑ (BCAA)
Mental performance ↓ (placebo)
Extracurricular activity = BCAA + placebo (substitution training times gave sig. difference)
No influence on extracurricular performance
Suppression protein degradation in BCAA group
No influence on extracurricular performance
No influence on extracurricular performance
No influence on extracurricular and endurance performance
QD (1 mg/kg): time to exhaustion ↓
LY 53857 (1.5 mg/kg): time to exhaustion ↑
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Abbreviation: AMPH = amphetamine; BCAA = branched chain amino acids; CHO = carbohydrate drink; DA = dopamine; exc = exercise; h = hours(s); i.g. = injection i.p. = intraperitoneal; LUMAA = large neutral amino acids; L-TRIP = L-tryptophan; LV 59857 = serotonin antagonist; mCPP = 5-HT(1A) agonist; 5-HTP = 5-hydroxytryptophan; QD = quipazine dimetrate; serotonin antagonist; RTE = run to exhaustion; s.c. = subcutaneous; S-D = Sprague-Dawley rats; sig. = significant; T = total; ↓ = decrease; ↑ = increase; = indicates no change; 8-OHDA = 5-hydroxydopamine; 8-OHDPAT = 5-HT(1A) agonist.

The above studies used field experiments (soccer match) to determine the effects of BCAA supplementation on performance. The results showed that BCAA supplementation improved performance in endurance events such as running and marathon runs. In the 30km trail run, there was a significant increase in muscle glycogen levels in the BCAA group compared to the placebo group.

The authors concluded that BCAA supplementation increased the availability of BCAAs, which improved performance. In addition, the BCAA supplementation increased the psychological response to a submaximal run, leading to better mood and reduced perceived exertion.

The studies also showed that BCAA supplementation improved performance in soccer matches, with players in the BCAA group showing improved performance compared to the placebo group. The improvement in performance was attributed to increased muscle glycogen levels and improved psychological response to submaximal runs.

A similar experiment studied the effects of BCAA supplementation in endurance events. The results showed that BCAA supplementation improved performance in the 30km trail run, with a significant increase in muscle glycogen levels in the BCAA group.

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logical responses to exercise and performance capacity.

Several other animal and human studies have examined this tryptophan- and serotonin-linked central fatigue hypothesis and the subsequent peripheral and central effects of endurance exercise. Davis et al. investigated the effects of carbohydrate feedings during prolonged exercise to fatigue on changes in plasma BCAAs, lactate, free fatty acids, insulin. These parameters could be important regulators of plasma tryptophan transport into the brain. The major finding of this study was that levels of plasma free tryptophan and free tryptophan/BCAAs and free fatty acids increased progressively during prolonged exercise until fatigue, while plasma BCAAs remained unchanged in the placebo group and decreased in the carbohydrate group. The changes in plasma free tryptophan, free tryptophan/BCAAs and free fatty acids were attenuated in a dose-dependent manner and fatigue was delayed when subjects consumed carbohydrate drinks.

Galliano et al. concluded that addition of small quantities of BCAAs to a typical sport drink may serve to maintain plasma BCAA levels throughout prolonged exercise, but does not appear to have any effect on psychological, endocrine, or performance responses during prolonged cycling. One study found that the supplementation of BCAAs suppressed the protein degradation during knee extension exercises for 60 minutes.

During exercise plasma levels of free tryptophan increase and it is proposed that this could be 'balanced' by raising the plasma level of BCAAs. Thus, according to the Newsholme hypothesis, supplementation with BCAAs will improve performance, while supplementation with tryptophan will have the opposite effect. This hypothesis was tested by several authors and the results indicate that oral supplementation with BCAAs or tryptophan significantly increases the plasma level of BCAAs. Neither time to exhaustion during cycling at 70 to 75% maximum oxygen uptake (VO₂max), nor performance during 100km cycling in well-trained cyclists differed. So, neither a positive effect of BCAA supplementation, nor a negative effect of tryptophan supplementation on performance was found.

Van Hall et al. investigated whether ingestion of tryptophan or BCAAs could influence performance. They calculated the transport of tryptophan (free or bound) into the brain during prolonged exhaustive exercise and found that the effect of BCAAs and tryptophan ingestion seems to be independent of whether total or free tryptophan is considered available for transport. Verger et al. examined the effects of administration of BCAAs versus glucose or water during acute exercise in the rat. They measured exercise time, blood insulin and glucose levels at exhaustion in the animals. The results showed that following ingestion of BCAA physical performance was lower and blood glucose levels between groups did not differ, while blood insulin level at exhaustion was higher with BCAAs than after glucose administration. It would have been interesting to have measured plasma free fatty acid level in this study and link this with the other parameters in order to get a more complete image of insulin, glucose, BCAAs and free fatty acid levels.

The decreases in BCAAs during prolonged exercise, as seen in the Blomstrand et al. studies, could also be the consequence of elevated plasma insulin levels. As previously mentioned, fluid intake was not totally controlled in these field studies, therefore it is likely that the study participants consumed carbohydrate-containing drinks. The effects of the above mentioned peripheral parameters and the link with insulin levels need further investigation since several studies have shown that not only insulin, but also amylin facilitates brain tryptophan uptake and monoamine metabolism.
Taken together these studies indicate that fatigue can be delayed by ingesting carbohydrates, which are an important energy source for muscle and brain function, and that until today there is little evidence to support the hypothesis that BCAA supplementation will increase performance.

3.2 Precursor Loading, Pharmacological Manipulation and Neurotransmission

The possibility of a centrally mediated fatigue during exercise was discussed by Romanovski and Gabriel[53] in the mid-1970s. They linked serotonin to a possible inhibition of brain oxidoreductive processes, while others[15,34,35,43] pointed out the role of dopamine in the onset of central fatigue. This brings us back to the possible interaction between neurotransmitters and their mutual influence on exercise performance. This was investigated by several authors who used precursor loading and/or pharmacological manipulation on exercise performance in animals and humans.

The effects of tryptophan supplementation on human performance were examined[84] and it was hypothesised that administration of tryptophan before exercise could contribute to a decreased sense of discomfort and pain associated with prolonged exercise. During a treadmill test at 80% VO\textsubscript{max}, the total exercise time to exhaustion increased by 49% in the tryptophan group, but this increase could have been confounded by a spectacular improvement in 2 of the 8 participants (160 and 260% increase in running time). This study was criticised by Chaouloff[89] and Stensrud et al.[75] who performed a similar study and found no differences between tryptophan and placebo groups.

Two studies[90,91] examined the effect of a serotonin reuptake inhibitor on time to exhaustion in humans. Both found a decrease in exercise time to exhaustion with the reuptake inhibitor compared to placebo. There were no differences in plasma glucose or lactate between both groups indicating the possible role of the central serotonergic systems in fatigue in people. De Meirleir[92] examined the influence of a dopamine agonist (pergolide) and a serotonin antagonist (ketanserin) on exercise performance. The results showed that oral treatment with the serotonin (5-HT\textsubscript{1C}/5-HT\textsubscript{2}) antagonist had no influence on exercise performance. It did not alter heart rate at rest or during exercise, but it elicited a shift to the right of the lactic acid curve.

The dopamine agonist (D\textsubscript{1}/D\textsubscript{2}) lowered heart rate, systolic blood pressure and enhanced maximal work capacity[92,93].

Chaouloff et al.[42,43,49,52] conducted a number of studies on the effects of tryptophan loading and/or exercise on central serotonin synthesis and metabolism. Under some pharmacological conditions running caused region-specific alterations in the conversion of tryptophan into the serotonin synthesis pathway.

The role of serotonin in the regulation of motor mechanisms is complex. Thus, a depletion of brain and spinal serotonin, as well as an increase in the availability of central serotonin, can result in a decrease or an increase in motor activity depending on the experimental model used.[94]

There are numerous levels at which central serotonin can affect motor behaviour, from sensory perception, sensory-motor integration to motor effector mechanisms. For reviews of the involvement of serotonin in the initiation or modulation of motor patterns, neuromuscular function and motor control, see Jacobs and Fornal,[6] Jacobs,[7] and Wallis.[86] Jacobs and Eubanks[86] measured the effects of serotonin and 5-HTP injections on motor activity and found it to decrease in a dose-dependent manner, with serotonin being a more potent inhibitor than 5-HTP. They attributed these behavioural changes to peripheral effects of serotonin. These results were not confirmed by Wilckens et al.,[3] who did not find any effect of serotonin administration on running wheel activity.

Another group[84] investigated the effects of systemically administered 8-OH-DPAT (a serotonin agonist with preference for the 5-HT\textsubscript{1A} binding site) on motor activity in open-field locomotion, and on treadmill running in rats. The spontaneous locomotor activity and rearing (vertical activity) were dose-dependently decreased by the administr-
tration of the 5-HT$_{1A}$ agonist. There were no statistically significant effects by 8-OH-DPAT on treadmill locomotion, i.e. the motor coordination was intact.\cite{117}

Chaouloff\cite{117} examined whether a short exercise training programme (4 days) could influence 5-HT$_{1A}$ receptor-mediated behaviours. The effects of 8-OH-DPAT administration were not affected by training or acute exercise.\cite{117} It should be mentioned that the administration of 8-OH-DPAT and other drugs including precursors, agonists and releasers, produces various signs of the so-called ‘serotonin syndrome’.\cite{7} This syndrome is characterised by hyperactivity, head shakes or ‘wet dog’ shakes, hyper-reactivity, tremor, rigidity, hindlimb abduction, Straub tail, lateral head weaving and reciprocal forepaw treading. These behavioural signs are sometimes used as an indication for central serotonin activity.\cite{7}

Wilckens et al.\cite{3} found with the same agonist (8-OH-DPAT) that locomotion increased during the first and second hour at the lowest dose and that at the higher doses locomotion was inhibited. This suppressed locomotion at higher doses could be a consequence of a behavioural impairment resulting from the ‘serotonin syndrome’.\cite{3} They further evaluated the effects of serotonin receptor agonists and antagonists with selectivity for various serotonin receptor subtypes on running wheel activity in the rat model for semi-starvation-induced hyperactivity, where running wheel activity was stabilised for 10 weeks at a high level of circa 20 to 25 km/day. The results showed that excessive running in the semi-starved rat is suppressed by activation of the 5-HT$_{1C}$ receptors and that activation of presynaptic 5-HT$_{1A}$ receptors resulted in a decreased serotonin release.\cite{3}

These results were confirmed by Bailey et al.\cite{44} who found a decreased run time to exhaustion in a dose-response manner in animals treated with mCPP (5-HT$_{1C}$ agonist). Other studies\cite{97,98} also suggested that hypolocomotion induced by mCPP could be mediated via postsynaptic 5-HT$_{1C}$ receptors.

That 5-HT$_{1C}$ receptors play a major role in the development of compulsive running is supported by the fact that the effect of the 5-HT$_{1C}$ receptor agonists on wheel running in rats could only be counteracted by serotonin antagonists which have high affinity for the 5-HT$_{1C}$ receptor (metergoline and mianserin).\cite{3} The inhibitory effect of the agonists on running wheel activity was prevented by pretreatment with antagonists that also had high affinity for the 5-HT$_{1C}$ receptors.\cite{3} Bailey et al.\cite{9,54} examined the effects of quipazine dimaleate (QD), a general serotonin agonist with high affinity for the 5-HT$_{3}$ receptor\cite{119} and LY53857, a serotonin antagonist specific to 5-HT$_{1C}$ and 5-HT$_{2}$ receptors. Run time to exhaustion was reduced in a dose-dependent manner by increasing dosages of quipazine dimaleate, while the time to exhaustion was increased with LY53857 administration but only at the highest dose.\cite{52} The results further indicated that QD appeared to block the increase in dopamine and DOPAC after 1 hour of exercise and LY53857 prevented the decrease in dopamine and DOPAC at fatigue,\cite{119} indicating the importance of the interaction between brain serotonin and dopamine in the onset of fatigue.

Pretreatment of exercising rats with amphetamine,\cite{99} a dopamine releaser, or apomorphine\cite{94} (a dopamine agonist), extends the time to exhaustion. Heyes et al.\cite{34} used the 6-hydroxydopamine (6-OHDA) model to induce a dopamine lesion and found these animals to have significant shorter run times to exhaustion. When apomorphine was given to 6-OHDA lesioned rats, their run time increased compared with saline-administered lesioned rats. Clonidine (noradrenaline receptor agonist) given to these animals had no effect.

These studies indicated that central dopamine depletion hastens time to exhaustion, while increasing central dopaminergic activity prolongs time to exhaustion.\cite{34} In a follow-up study the same authors\cite{35} found an increase in both dopamine synthesis and release with exercise. Striatal dopamine depletion had no effect on the rats ability to run during the first 40% of an exhaustion run, but accelerated the deterioration in exercise perfor-
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mance during the remaining 60% of the run and hastened exhaustion.

The results of these studies suggest that the time to exhaustion is influenced by the activity of dopaminergic neurons. During the later phase of the run to exhaustion there is probably a need to increase striatal dopaminergic activity. If this is the case, dopaminergic agonists may improve exercise capacity by facilitating such recruitment. On the other hand, these authors state that dopamine accumulation in the striatum late in exercise as found in their studies may reflect decreases in dopamine release, perhaps due to activation of dopamine autoreceptors.

A low dose of haloperidol (dopamine antagonist) disrupts the treadmill performance and Chaouloff et al. reported that when haloperidol was administered at the beginning of exercise the animals were unable to run. The injection of haloperidol at the end of exercise caused a large increase in DOPAC in the brains of controls and runners.

The results from these studies emphasise the importance of dopamine and serotonin (and probably other neurotransmitter) interactions during exercise. Central 5-HT₃ receptors might be involved in the observed behaviour because they interact with the dopaminergic neurotransmitter system. LY53857 is a potent and selective 5-HT₃ receptor antagonist, but has also affinity for α₂ receptors, therefore at the highest dose can interact with catecholaminergic receptors since Wilckens et al. found that propanol increased motor activity in the rats, which could arise from the action of this serotonin antagonist (5-HT₁A, B, C) on β-adrenoceptors.

Taken together, these results indicate a role for several dopamine and serotonin transmitter receptors in motor control and the so-called ‘central fatigue’, but as Bailey and Davis pointed out: any possible role of serotonin, dopamine (and other transmitters) in motor function should be perceived as a continuum. This continuum is not only important at the brain level, but has its own importance in the interaction between central neurotransmission and the peripheral processes during exercise, including the neuro-endocrine system, especially the activity of the hypothalamic-pituitary-adrenal (HPA) axis. Neurotransmitter systems not only influence each other, but they are also intimately linked to the HPA axis. We will not include an overview of the interactions between stress hormones and central neurotransmitter systems, for a review on serotonin and stress hormones see the excellent review of Chaouloff.

4. Measurement of Extracellular Neurotransmitter Levels

The review of the literature demonstrates that serotonergic, noradrenergic and dopaminergic neuronal systems are influenced in different ways during exercise. However, most of the studies were post mortem experiments which used indirect measurements such as the ratio of neurotransmitter to metabolites, or precursor to neurotransmitter to predict neurotransmitter release during exercise. Changes in the brain content of monoamine transmitters with tissue assay are now regarded as a rather inaccurate method to estimate changes in the release rate of these transmitters. Recently, new techniques such as microdialysis and voltammetry were introduced to measure in vivo release of neurotransmitters (see table V).

The voltammetry method is based on the application of a potential to an electrode in a conducting solution. The electrodes are implanted in the brain and an oxidation current is generated as molecules in the extracellular fluid are oxidised at the electrode surface. Microdialysis is a means of assessing alterations in neurotransmitter release in brain extracellular space. It can collect virtually any substance from the brains of freely moving animals with a limited amount of tissue trauma. This method allows the measurement of local neurotransmitter release in combination with on-going behavioural changes such as exercise. A number of microdialysis studies have documented changes in extracellular neurotransmitters in several brain areas dur-
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Abbreviations and symbols: Ach = acetylcholine; DA = dopamine; DHPG = 3,4-dihydroxyphenylglycol; DOPAC = 3,4-dihydroxyphenylacetic acid; exc = exercise; GABA = γ-aminobutyric acid; GIL = glutamic; h = hour(s); HVA = homovanillic acid; MHPG = 3-methoxy-4-hydroxyphenylethylglycol; NA = noradrenaline (norisophrine); S-D = Sprague-Dawley rats; sig. = significant; TTL = total; ↑ = increase; ↓ = decrease; = no significant difference.
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Fig. 3. The effects of 20 minutes of exercise on extracellular monoamine levels in rat striatum (after Meuson et al. 1994[96], with permission). Compared to baseline values, the brain monoamine release increased during 20 minutes of exercise. Maximal extracellular levels were obtained 20 minutes post-run for dopamine, while noradrenaline (norepinephrine) and serotonin (5-hydroxytryptamine; 5-HT) values peaked during running. After this increase extracellular levels of the neurotransmitters remained above baseline for at least 60 to 80 minutes, while serotonin remained elevated up to 120 minutes after the exercise was stopped. Abbreviations: DA = dopamine; NA = noradrenaline (norepinephrine).

Recently, the first reports appeared, demonstrating that it is possible to measure extracellular levels of neurotransmitters with microdialysis in the rat brain during exercise and recovery from exercise. Two studies[55,112] found that 20 minutes of exercise on a treadmill, significantly increased dopamine release in rat striatum. Hattori et al.[112] combined microdialysis with running in order to evaluate motor deficit and improvement following dopaminergic grafts in 6-OHDA lesioned rats. Dopamine, DOPAC and homovanillic acid significantly increased during the treadmill exercise in their control animals.[112]

In another study a circular treadmill (speed of the treadmill circa 10 m/min) was used in order to let the animals turn and ‘walk in place’ for sucrose water reward.[113] The rats were fixed by their tails and walked in place for 24 minutes. Extracellular levels of dopamine and DOPAC were measured in the nucleus accumbens.medial striatum and lateral striatum. An increase in dopamine, and DOPAC release in the lateral striatum was found. The authors were unable to conclude whether these changes were due to motor activity, the act of drinking, the tail pinch stress or the amount of fluid consumed.

Another study registered the simultaneous release of monoamines in rat striatum, during and following exercise, and found a significant increase of these neurotransmitters (fig. 3).[55] In a similar experiment[114] it was demonstrated that a light exercise regimen is able to significantly increase extracellular levels of glutamate (GLU), while γ-aminobutyric acid (GABA) remains unchanged (fig. 4). These results could indicate the existence of a functional interaction of several brain neurotransmitters in the regulation of locomotion.
Kurosawa et al.\textsuperscript{[57]} used a treadmill that was manipulated manually at a low speed (2.3 m/min). The animals were restrained in a metal harness which was fixed to the rat’s chest and abdomen with plaster. Extracellular acetylcholine (ACh), noradrenaline and serotonin in the parietal lobe of the cerebral cortex was examined. Walking for 5 minutes produced an increase of all 3 neurotransmitters.

Treadmill running for 60 minutes significantly increased extracellular serotonin level in the hippocampus of trained rats.\textsuperscript{[56]} Pagliari et al.\textsuperscript{[111]} examined the effect of exercise on the in vivo cerebral release and turnover of noradrenaline in trained rats running on a treadmill for 60 minutes. The authors used a chronic probe implantation in the frontal cortex. Noradrenaline turnover and release increased during exercise and even further increased when exercise time was prolonged to 2 hours of running.

Gerin et al.\textsuperscript{[115]} used an interesting approach. To investigate the effects of exercise on spinal cord serotonin, these authors chronically implanted a microdialysis probe in the ventral horn of the lumbar spinal cord of rats. The probe was kept in place during 40 days. Extracellular release of serotonin did not increase during 60 minutes of exercise.

Two studies\textsuperscript{[22,116]} used in vivo voltammetry. Bertolucci-D’Angio et al.\textsuperscript{[22]} studied the dopamine metabolism in different brain regions and compared forced locomotion with several other stressors. Forced locomotion of rats on a rotarod for 40 minutes increased the amplitude of the DOPAC oxidation peak in the striatum and the nucleus accumbens, but failed to affect the DOPAC peak in the prefrontal cortex. This increase in the striatum and nucleus accumbens is compatible with the currently held view that the nigrostriatal dopaminergic neurons are associated with motor function.\textsuperscript{[22]}

In addition to these results Guadeloupe et al.\textsuperscript{[116]} investigated the effects of forced locomotion and spontaneous locomotion on dopamine and DOPAC in the nucleus accumbens. They found that continuous and intermittent locomotion increased the levels of dopamine and its metabolite indicating the involvement of the nucleus accumbens in the
initiation but not the maintenance of movement.\textsuperscript{116}

The recently developed and more sophisticated \textit{in vivo} methods such as microdialysis and \textit{in vivo} voltammetry will improve our insight into the relationship between the monoamine and other transmitters during on-going behaviour such as exercise. These methods will allow us to monitor extracellular release and metabolism of various neurotransmitters. However, the few studies that have been published up till now, already show the same discrepancies as in the post mortem studies. They used different exercise models, for example, with or without extra stress (electrical grid at the end of the treadmill, restraint in a harness), other running speeds and training regimens. We therefore hope that in the future these precise collection methods, will be used in well-defined experimental protocols in order to being able to compare the different results.

\section*{5. Conclusions}

There is consensus that monoaminergic neurons are involved in a number of functions that regulate locomotion. Although most studies used different experimental protocols, it can be concluded that brain neurotransmission is influenced by exercise. The effects of exercise on neurotransmission should be explored in a multidimensional way because there is a constant interaction between several neurotransmitters and their respective receptors during locomotion.

Many neurotransmitters or neuromodulators influence an individual’s ability to exercise via actions in both the peripheral and central nervous system. The intracerebral mechanisms responsible for the central fatigue phenomenon have not been fully identified. Animal and human studies will help us to find out the effects of various pharmacological manipulations on central fatigue.

It would be of interest to study whether the different neurotransmitter interactions can influence ‘central fatigue’ during exercise. We need to develop standard experimental strategies in order to examine the effects of exercise on brain functions.

Neurotransmitter inter-relationships are important since these interactions reflect the multidimensional image of the different processes that happen in the brain during exercise. \textit{In vivo} methods will allow us to explore the interaction between neurotransmitters and receptors and get more insight into areas such as neurotransmitter release, metabolism, reuptake and receptor sensitivity.

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\section*{References}

3. Wäckens T, Schweiger U, Petke K. Activation of 5-HT\textsubscript{1C} receptors suppresses excessive wheel running induced by sedation in the rat. Psychopharmacol 1992; 109: 77-84


90. Wilson W, Maughan R. Evidence for a possible role of 5-hydroxytryptamine in the genesis of fatigue in man: administr...