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## **Anatoxin-a: overview on a harmful cyanobacterial neurotoxin from the environmental scale to the molecular target**

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## Abstract

Anatoxin-a (ATX-a) is a neurotoxic alkaloid, produced by several freshwater planktonic and benthic cyanobacteria (CB). Such CB have posed human and animal health issues for several years, as this toxin is able to cause neurologic symptoms in humans following food poisoning and death in wild and domestic animals. Different episodes of animal intoxication in the wild have incriminated ATX-a, as confirmed by the presence of ATX-a-producing CB in the consumed water or biofilm and/or the observation of neurotoxic symptoms, which match experimental toxicity *in vivo*. Regarding toxicity parameters, toxicokinetics knowledge is currently incomplete and needs to be improved. The toxin is able to cross passively biological membranes and act rapidly on nicotinic receptors, its main molecular target. *In vivo* and *in vitro* acute effects of ATX-a have been studied and make possible to draw its mode of action, highlighting its deleterious effects on the nervous systems and its effectors, namely muscles, heart and vessels, and the respiratory apparatus. However, very little is known about its putative chronic toxicity. This review updates available data on ATX-a, from the ecodynamic of the toxin to its physiological and molecular targets.

Keywords: cyanobacteria · anatoxin-a · nicotinic acetylcholine receptor · nervous system · algal blooms

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## 1. Introduction<sup>1</sup>

In the context of global changes, with - among other consequences - the increase of N and P inputs due to agricultural activities, and the rise of temperatures and low-water periods, the phyto-benthic and phytoplanktonic communities are largely dominated by cyanobacteria (CB) (O’Neil et al., 2012).

One of the deleterious consequences of the rapid proliferation of CB, often due to weak water circulation, is the production and secretion of cyanotoxins which develop a large range of toxicity mechanisms, the main ones being hepatotoxicity, dermatotoxicity and/or neurotoxicity caused by hepatotoxins (eg microcystins, nodularins), dermatotoxins (eg lyngbiatoxins) and/or neurotoxins (anatoxins, saxitoxins), respectively (Sivonen and Jones, 1999; Osswald et al., 2007; Aráoz et al., 2010; Huang and Zimba, 2019). However, the ecological role of these toxins, in terms of biological benefits for the CB, remains still poorly understood. Some of the possible roles explored in the literature are (i) to limit CB predation by other organisms such as zooplankton and nekton, and/or (ii) to serve as chemical mediators involved in cellular communication – allelopathy and chemotaxis – which can establish ecological relationships with other CB or other micro- or macro-

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<sup>1</sup>Abbreviations used: ACh Acetylcholine; ATX-a Anatoxin-a; CB Cyanobacteria; CNS Central nervous system; dhATX-a Dihydroanatoxin-a; dhHTX-a Dihydrohomoanatoxin-a; FW Fresh weight; HTX-a Homoanatoxin-a; IP Intraperitoneal; IV Intravenous; LD<sub>50</sub> Lethal dose 50%; LD<sub>90</sub> Lethal dose 90%; LOAEL Lowest observed adverse effect level; mAChR Muscarinic acetylcholine receptor; MC Microcystin; nAChR Nicotinic acetylcholine receptor; NOAEL No observable adverse effect level; PNS Peripheral nervous system; PKS Polyketide synthase; TE Thioesterase

1 organisms and reduce the development of competitors (Kaebernick and Neilan,  
2 2001; Holland and Kinnear, 2013).  
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8 **Figure 1 near here**  
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15 In freshwater, the significant concentrations of cyanotoxins are consecutive to the  
16 proliferation of cyanotoxin-producing CB. Anatoxin-a (ATX-a) and related molecules  
17 are mainly secreted by freshwater benthic CB (Figure 1) (e.g., *Phormidium*,  
18 *Kamptonema*, *Microcoleus*, *Oscillatoria*) in weak water currents (Edwards et al., 1992  
19 ; Gugger et al., 2005 ; Wood et al., 2018), and planktonic CB (e.g., *Anabaena*,  
20 *Aphanizomenon*, *Cuspidothrix*, *Cylindrospermopsis*, *Cylindrospermum*,  
21 *Dolichospermum*, *Planktothrix*, *Raphidiopsis*, *Tychonema*) in stagnant water  
22 (Sivonen et al., 1989 ; Viaggiu et al., 2004 ; Vehovszky et al., 2009 ; Hodoki et al.,  
23 2013 ; Shams et al., 2015 ; Trainer and Hardy, 2015 ; Ballot et al., 2018) . This  
24 harmful cyanotoxin causes neurotoxic symptoms, sometimes lethal, in livestock, fish,  
25 pets and humans (Osswald et al., 2007). (Osswald et al., 2007).  
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## 48 **2. Chemistry of ATX-a**

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52 ATX-a (2-acetyl-9-azabicyclo[4.2.1.]non-2-ene – MW 165 Da, C<sub>10</sub>H<sub>15</sub>NO) is a water-  
53 soluble secondary bicyclic amine, structurally related to alkaloids (Figure 2). It mainly  
54 exists in cationic form (pKa 9.6) and proved to be photosensitive at alkaline pH,  
55 where it degrades into non-toxic compounds (see section 3.2; Stevens and Krieger,  
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1991). The natural isomer is (+)-ATX-a, and the production of (-)-ATX-a by cyanobacteria has not been described to date. In this review, we specify - when available - which toxin isomer is used experimentally.

**Figure 2 near here**

ATX-a should not be confounded with ATX-a(s), which is also a neurotoxic cyanotoxin, but structurally related to organophosphates. ATX-a(s) will not be considered in this review. Several analogues of ATX-a have been detected in different field samples or in CB cultures (Skulberg et al., 1992; James et al., 1998). The most common are homoanatoxin-a (HTX-a), dihydroanatoxin-a (dhATX-a) and dihydrohomoanatoxin-a (dhHTX-a) (Figure 2). HTX-a (2-(propan-1-oxo-1-yl)-9-azabicyclo [4.2.1] non-2-ene – MW 179 Da, C<sub>11</sub>H<sub>18</sub>NO) is a methylated homologue of ATX-a with a comparable toxicity (Devlin et al., 1977; Wonnacott et al., 1992; Osswald, 2007; Aráoz et al., 2010), whereas dhATX is ten times less potent than ATX-a (Wonnacott et al., 1991). These four congeners can be found simultaneously in the environment in variable proportions. According to recent studies, dhATX-a seems to be the most common congener in New Zealand rivers followed by dhHTX-a (McAllister et al., 2016; Wood et al., 2018).

### 3. Biosynthesis and transformation

#### 3.1. Biosynthesis

1 On the biosynthesis of ATX-a and HTX-a, a partial pathway using glutamate as  
2 substrate and involving polyketide synthases (PKS) was firstly described  
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4 (Hemscheidt *et al.*, 1995). As such, the production of ATX-a results from the  
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6 condensation to three units of acetate on the (S)-1-pyrroline-5-carboxylate, which  
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8 derived from glutamate, followed by a decarboxylation. HTX-a is thought to result  
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10 from the consecutive methylation of ATX-a. Thereafter, the genes encoding PKS  
11  
12 enzymes involved in ATX-a biosynthesis have been identified (Cadel-Six *et al.*,  
13  
14 2009). A nucleotidic sequence of *Oscillatoria* sp. strain PCC 6506 encoding a PKS  
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16 has been found only in the genome of strains producing ATX-a or HTX-a, and  
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18 surprisingly, within a cluster of genes encoding probably various enzymes involved in  
19  
20 the biosynthesis of secondary ATX-a metabolites (Méjean *et al.*, 2010).  
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22 Bioinformatics analysis of the cluster showed that these genes encoded three  
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24 proteins – namely AnaB (Prolyl-ACP oxidase), AnaC and AnaD – which are involved  
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26 in the production of a substrate for three PKS enzymes (AnaE, AnaF, AnaG).  
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28 Predicting the function of each of these gene products has allowed the description of  
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30 a full biosynthetic pathway for ATX-a and HTX-a (Méjean *et al.*, 2009, 2014). The  
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32 cyclic amino acid proline is activated and attached to an acyl-carrying protein (AnaD)  
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34 by an adenylation protein (AnaC). Then, the oxidation of this prolyl-ACP to 1-  
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36 pyrroline-5-carboxyl-ACP (P5CACP) is performed by the AnaB oxidase. This newly  
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38 formed imine is processed by a first PKS (AnaE), which adds a reduced acetate unit.  
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40 The chain is then modified by the second PKS (AnaF), which probably adds an  
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42 acetate unit and performs a Michael-type cyclization leading to the anatoxic  
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44 thioester linked to the ACP domain. The latest PKS (AnaG) is supposed to add an  
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46 unreduced acetate unit. Subsequently, methylation is carried out to form the  
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48 precursor of HTX-a. Without this methylation step, the latter PKS is the precursor of  
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ATX-a. In fact, since the latter PKS does not have a thioesterase (TE) domain, TE type II (AnaA) performs hydrolysis to form carboxyATX-a or carboxy-homoATX-a, which lead to the corresponding compounds through spontaneous decarboxylation (Méjean et al., 2014).

### 3.2. Transformation

ATX-a and HTX-a are unstable in natural condition, where they are partially or totally degraded and converted into low- or non-toxic metabolites dhATX-a and epoxyATX-a (Stevens et Kriger, 1991; James et al., 1998). ATX-a can be degraded through photolysis and non-photochemical reactions, each of them affecting its chemical stability. It is also less bioavailable by dilution and adsorption (Stevens and Krieger, 1991). ATX-a degrades more quickly at higher temperatures (-76% after 1-hour exposure at 100°C, pH 7) and alkaline pH (-48% after 60-days exposure at pH 9.5°C, 20°C) (Stevens and Krieger, 1991; Kaminski et al., 2013). Based on analysis of reaction products by gas chromatography-chemical ionization mass spectrometry (GC/MS) from various experimental photolysis, several products were identified weighing 165 Da, corresponding to the MW of ATX-a. It was concluded that photolysis of ATX-a generated a complex mixture of rearrangement products involving different bridgehead positions (Stevens and Krieger, 1991; Kaminski et al., 2013). The breakdown products resulting from both photolytic and non-photolytic degradation are less toxic than ATX-a, some even being inactive (Stevens and Krieger, 1991). However, UV-B irradiation and high temperatures degraded ATX-a in the same way, leading to similar metabolites (Afzal et al., 2010; Kaminski et al., 2013). Its half-life in freshwater is estimated to be 5 days at neutral pH (Smith and



1 Sutton, 1993), but under conditions of sunlight intensity and at pH 9, ATX-a has a  
2 half-life of 1-2 h (Stevens and Krieger, 1991).  
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5 Micro-organisms are also able to degrade ATX-a, in different type of lake sediments  
6 which undergo cyanobacterial blooms, but also in lake sediments with no history of  
7 proliferation. In the Finnish eutrophic Lake Tuusulanjärvi the toxin concentration in  
8 water drops from 2.4  $\mu\text{g}\cdot\text{mL}^{-1}$  to 0 within 4 days (Rapala et al., 1994). It also has  
9 been shown that *Pseudomonas* sp. degrades ATX-a at a rate of 6-30  $\mu\text{g}\cdot\text{mL}^{-1}$  per 3  
10 days (Kiviranta et al., 1991).  
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#### 23 **4. Factors influencing ATX-a production**

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27 Environmental conditions have a significant impact on cyanotoxin biosynthesis  
28 (Neilan et al., 2013; Boopathi and Ki, 2014). The amount of toxin can be up-regulated  
29 through an increase of the CB biomass, and/or an increase in toxin biosynthesis.  
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31 Both mechanisms have been observed simultaneously.  
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38 Average concentrations of ATX-a measured range from 0.1 to 0.6  $\mu\text{g}/\text{L}$  of water  
39 samples (Cerasino and Salmaso, 2012). These concentrations can somehow reach  
40 much higher levels such as 172,640  $\mu\text{g}\cdot\text{L}^{-1}$  in 2008 in the Anderson lake (USA)  
41 (Hamel, 2009). In CB biofilms, ATX-a concentrations reach 1233  $\mu\text{g}\cdot\text{g}^{-1}$  dw in the  
42 Tarn River (France) and 712  $\mu\text{g}\cdot\text{g}^{-1}$  in the Oreti River (New Zealand) (McAllister et al.,  
43 2016; Echenique-Subiabre et al., 2018). In natural conditions, the cellular  
44 concentration of ATX-a was 2.5  $\text{pg}\cdot\text{cell}^{-1}$  (Wood and Puddick, 2017). This cell  
45 content of ATX-a can reach maximal values and 1683  $\mu\text{g}\cdot\text{g}^{-1}$  dw, and a production of  
46 146  $\mu\text{g}\cdot\text{L}^{-1}$  in an *Aphanizomenon issatschenkoi* strain under moderate levels of  
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1 nitrogen (Gagnon and Pick, 2012), but experimental conditions influence this level. A  
2 23-day culture of the same CB ended up with a maximum of 1105  $\mu\text{g.L}^{-1}$  production  
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4 (Selwood et al., 2007). These values, among others, illustrate the heterogeneous  
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6 nature of ATX-a production.  
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10 Early studies demonstrated that light, temperature and growth phase influenced ATX-  
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12 a production by *Anabaena flos-aquae* NRC-44-1 strains (Peary and Gorham, 1966).  
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14 The highest toxicity was observed after 14 growing days at 22°C and 2000-7500 lux.  
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16 Similar results confirmed the relationship between physicochemical parameters,  
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18 growth phase and ATX-a production by cells (Rapala et al., 1993; Rapala and  
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20 Sivonen, 1998). The amount of ATX-a produced by cell appears to be correlated with  
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22 the exponential growth phase (Gagnon and Pick, 2012). In fact, the highest ATX-a  
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24 intracellular levels are found during the first two weeks of CB growth before reaching  
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26 a plateau and halving within a week (Rapala et al., 1993; Gallon et al., 1994; Harland  
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28 et al., 2013).  
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35 In addition, the synthesis of ATX-a has been shown to be down-regulated at high  
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37 temperatures, regardless of cyanobacterial cell growth, in *Anabaena* and  
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39 *Aphanizomenon* strains (Rapala et al., 1993). Temperature has a significant impact  
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41 on toxin concentrations. The highest intracellular ATX-a concentrations have been  
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43 recorded between 19°C and 21°C (Rapala et al., 1993; Rapala and Sivonen, 1998).  
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45 When temperatures rise too much, ATX-a production decreases to a minimum  
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47 observed at 30°C (Rapala et al., 1993). N and P concentrations also influence the  
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49 production of ATX-a. It is suggested that cellular production of ATX-a may be  
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51 stimulated by moderate N deficiency (Gagnon and Pick, 2012). Besides, the  
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53 available form of N may also play a role in ATX-a production. The toxin concentration  
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55 is slightly higher when CB are in a medium with  $\text{N}_2$  or  $\text{NH}_4\text{-N}$  compared to a medium  
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1 enriched with NO<sub>3</sub> or urea-nitrogen. However, no correlation between levels of toxin  
2 produced by CB and P concentrations has been observed. A deficiency in P seems  
3 only to limit the cyanobacterial growth (Rapala et al., 1993).  
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7 In addition to influencing toxin production, temperature and environmental  
8 composition also modulate the preferential biosynthesis of ATX-a or HTX-a (Aráoz et  
9 al., 2005). For instance, when grown on BG11 medium at 22°C without CO<sub>2</sub>  
10 enrichment, *Oscillatoria* PCC 6506 preferentially produces ATX-a. Conversely, when  
11 grown in BG1110 (with 10 nM NaHCO<sub>3</sub>) at 25°C with CO<sub>2</sub> enrichment, HTX-a is the  
12 dominant congener. Different strains of the same species may respond in multiple  
13 ways to the same environmental conditions. Two strains of *Oscillatoria* sp. (PCC  
14 6506 and PCC 9029), although deriving from the same original isolate, exhibit  
15 different toxin production profiles. Strain PCC 6506, grown under standard conditions  
16 in non-gassed flasks, in both BG11 and 2N media, exclusively produced ATX-a. In  
17 contrast, PCC 9029 grown under the same conditions, produced mainly HTX-a  
18 (Aráoz et al., 2005).  
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37 Finally, biotic interactions can also influence the production of ATX-a, or at least the  
38 abundance of CB possessing the *ana* gene cluster. Microbial assemblages in mat  
39 samples with the ATX-a biosynthetic gene cluster consistently present a lower  
40 relative proportion of *Burkholderiales* (Betaproteobacteria) species than did mats  
41 without ATX-producing genes, suggesting that certain non-cyanobacterial organisms  
42 select CB species with genes for toxin production (Bouma-Gregson et al., 2019). In  
43 conclusion, abiotic and biotic parameters seem to play a crucial role in toxigenic CB  
44 biomass and toxin production.  
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## 5. Bioaccumulation and depuration

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3 There is still very little work on the accumulation and excretion of ATX-a, so it does  
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5 not provide a clear picture. Although ATX-a is found in the environment – water and  
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7 biofilms samples –, naturally exposed organisms do not seem so far to prominently  
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9 bioaccumulate the toxin (Osswald et al., 2007; Ferrão-Filho and Kozlowsky-Suzuki,  
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11 2011). However, it is important to point out that the hypothesis of ATX-a  
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13 bioaccumulation is recent and has been little explored. Toxicokinetics in fish appears  
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15 to be fairly rapid. Following oral exposure of adult female medaka fish to a No  
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17 Observable Adverse Effect Level (NOAEL = 6.67  $\mu\text{g}\cdot\text{g}^{-1}$  of ( $\pm$ )-ATX-a), the toxin could  
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19 not be detected in the liver after 12h, and in the gut and muscles after 3 days (Colas  
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21 et al., 2020). Besides, the depuration rate for the 12 first hours is 57, 100 and 90% in  
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23 guts, livers and muscles, respectively, leading to the rapid excretion of ATX-a. This  
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25 suggests that fish do not bio-accumulate ATX-a in these tissues.  
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33 In 2009 and 2010, environmental and biological samples (water, fish, plants) were  
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35 investigated for the presence of ATX-a in various watering places in Nebraska and  
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37 Washington (USA). Extracellular ATX-a was found but no trace detected in all fish  
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39 samples studied, while other cyanotoxins – namely  $\beta$ -N-methylamino-L-alanine  
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41 (BMAA) and 2,4-diaminobutyric acid dihydrochloride (DABA) – were present in the  
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43 same analysed tissues (Al-Sammak et al., 2014; Hardy et al., 2015). When testing  
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45 the ability of the rainbow trout (*Oncorhynchus mykiss*) to bioconcentrate ATX-a by  
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47 balneation in water containing dissolved ATX-a up to 5  $\text{mg}\cdot\text{L}^{-1}$ , no significant  
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49 difference between bioconcentration factors (BCF) of ATX-a in juvenile subjects  
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51 exposed to different doses of the toxin for 96 h was observed: in fact, ATX-a  
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53 accumulates in fish body with BCF ranging from 30 to 47 (Osswald et al., 2011).  
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1 The exposure of the mussel *Mytilus galloprovincialis* to cultures of ATX-a-producing  
2 CB for 15 days revealed very low assimilation rates, despite the great capacity of this  
3 bivalve to bioaccumulate different toxicants, such as heavy metals, drug residues or  
4 even microcystins (MCs) (Vasconcelos, 1995; Osswald et al., 2008). Moreover, only  
5 one day after being placed in a CB-free environment, mussels presented no  
6 detectable ATX-a in their tissues. This toxin, which was mainly found in the digestive  
7 tract, seems to be quickly eliminated within 24h.  
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17 Under natural conditions, ATX-a was detected for the first time in fish harvested in a  
18 Polish lake by Pawlik-Skowrońska et al. (2012). The highest amounts were mainly  
19 found in livers compared to muscles, omnivorous fish accumulating roughly the same  
20 amounts of ATX-a as carnivorous fish: omnivorous roach (*Rutilus rutilus*) exhibited  
21 concentrations of 11 to 18 µg/g FW in liver in autumn 2008. Moreover, the bream  
22 *Abramis brama* from a dam reservoir was found to accumulate 6.2 to 18.4 µg/g FW in  
23 liver in 2010 (Pawlik-Skowrońska et al., 2013). The contents of ATX-a detected in  
24 fish tissues seemed to increase with the occurrence of the toxin in the environment.  
25 Both intracellular and extracellular ATX-a were found and the intracellular form was  
26 predominant, i.e. 10 times in spring and 3 times in summer (Pawlik-Skowrońska et  
27 al., 2013).  
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45 ATX-a has also been detected in aquatic plants of the genus *Myriophyllum* (Al-  
46 Sammak et al., 2014). This contamination may be due to the endosymbiotic  
47 relationships of CB with some other higher plants, but may also occur via the  
48 presence of cyanotoxins in the aquatic environment and their absorption by vegetal  
49 organisms (Al-Sammak et al., 2014). Other aquatic organisms were challenged for  
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ATX-a bioaccumulation, such as benthic *Chironomus* which, fed on MC- and/or ATX-a-producing CB, accumulated cyanotoxins (Toporowska et al., 2014).

## 6. Accidental exposure

### 6.1. Animal intoxications

Aquatic organisms can be naturally exposed to ATX-a by the oral, dermal or respiratory route (Kuiper-Goodman et al., 1999; Carmichael et al., 2001; Draisci et al., 2001). The ingestion of cyanotoxin-contaminated water is the cause of frequent cases of acute animal intoxication causing neurologic symptoms up to death (Quiblier et al. 2013, Wood et al, 2020). Water originating from a source with a proliferation of CB may contain toxins released during cell lysis (e.g. MCs) or excreted by CB (e.g. ATX-a) (Chorus and Bartram, 1999). Acute risks to animal health increase with the direct ingestion of CB biomass or biofilms, or of water containing large quantities of cyanotoxins. Alternatively, chronic intoxication may happen with the frequent ingestion of small doses of cyanotoxins over a long period of time through drinking water or food consumption (Svirčev et al., 2010). The irrefutable imputation of a poisoning to a single toxin often lacks evidence because the same species of CB can produce different kind of toxins. For instance, *Anabaena spiroids* produces ATX-a and also MCs; some *Kamptonema* species can produce both ATX-a and cylindrospermopsin (Méjean et al., 2009; Mazmouz et al., 2010).

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Nevertheless, ATX-a has been implicated in numerous animal poisonings worldwide (Table 1). Notably, the death of dogs due to ATX-a poisoning has been reported in North America, in New Zealand, but also in France, Kenya and Germany among others (Edwards et al., 1992; James et al., 1997; Hamill et al., 2001; Gugger et al., 2005; Wood et al., 2007; Pushner et al., 2008, 2010; Faassen et al., 2012; Fastner et al., 2018). In addition, ATX-a and ATX-a-secreting CB have been found in tanks used for domestic water supply (Fawell et al, 1999).

## 6.2. Human intoxication

The presence of cyanotoxins in drinking water is not uncommon and has been mentioned in Asia, Europe and Oceania in the last ten years (Buratti et al., 2017). The consumption of cyanotoxin-containing water poses a health issue as it arises acutely in regions where the supply of drinking or irrigation water is made by storage – notably dams, reservoirs, wells, retention basins – likely to accumulate toxin-producing CB or cyanotoxins, and no or insufficient treatment of drinking water. Human intoxications might happen through the consumption of water, but other routes of exposure could be implicated.

With only one exception (Biré et al., 2020), there is no epidemiological data on human poisoning formally incriminating ATX-a, although in several cases it is suspected to be the cause of symptoms in humans, due to their neurotoxic type (Stewart et al., 2006; Weirich and Miller, 2014). Human accidental ingestion of water during aquatic activities may result in the ingestion of dissolved cyanotoxins or cyanotoxin-producing CB. A variety of symptoms have been described, in relation to cyanotoxin exposure through water drinking.

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The time to onset of symptoms - depending on the toxin involved, its dose and the route of exposure - are highly variable: a few minutes or even a few hours for skin symptoms and neurological disorders, several hours for hepatotoxins. The most commonly reported symptoms are gastrointestinal signs (diarrhoea, vomiting), fever and skin irritations. Headache, sore throat, and dry cough can be observed. Considering only the ingestion or inhalation of CB, hepatic toxicities and neurotoxicities are more frequently described (Wood, 2016; Svirčev et al., 2019).

Despite this risk associated with such intoxication, no recurrent cases of human death associated with swimming have been reported. The only exception occurred in July 2002, following a swim in water with a bloom of ATX-a producing CB, in Wisconsin in the United States: five teens developed gastrointestinal symptoms 48 hours after swimming, and one died from heart failure. Stool and blood tests revealed the presence of *Anabaena flos-aquae* CB. ATX-a has been incriminated, despite the delay between exposure and death. However, this case has not been the subject of a scientific report (Weirich and Miller, 2014), and the identification of ATX-a was subsequently questioned (Carmichael et al., 2004; James et al., 2005). To date, there is only one human poisoning case formally involving ATX-a: the consumption of ascidians of the *Microcosmus* genus in the south of France which intoxicated 26 people. The symptoms observed, mainly neurotoxic (blurred vision, ataxia, dizziness, asthenia, headache, muscle cramps, paresthesia) are entirely compatible with exposure to ATX-a. This work identified ATX-a at concentrations of 193.7 to 1240.2  $\mu\text{g} \cdot \text{kg}^{-1}$  in the seafood consumed (Biré et al., 2020).

## 7. Toxicity



## 7.1. Acute toxicity

The symptoms observed experimentally after acute exposure to ATX-a are predominantly neurotoxic (Jakubowska and Szeląg-Wasielewska., 2015). There are no formal kinetic data for ATX-a in mammals. However, toxicity studies indicate that the toxin is rapidly absorbed after ingestion and widely distributed in highly vascularized organs such as liver, gut and muscles. Because it is a secondary amine, it rather crosses the blood-brain barrier in rats, which contributes to its acute toxicity, namely a decrease of locomotor activity (Rowell and Wonnacott, 1990; Stolerman et al., 1992).

**Table 2 near here**

ATX-a generally causes paralysis of intoxicated organisms. In rodents, ATX-a is lethal for doses of about 0.1 to 15 mg.kg<sup>-1</sup>, depending on the route of exposure and the enantiomer tested (Table 2). In mice, ATX-a IP exposure causes after few minutes' hyperventilation, tremors, mild convulsions, urination, hypersalivation, hyperactivity, muscle contractions, then a decrease in locomotor activity, muscle paralysis and respiratory arrest preceding death. The acute toxicity shows rapid effects (2 to 6 min after administration) with muscular and respiratory paralysis. At a sub-lethal dose, there is a retrocession of all symptoms observed. However, and although animals can recover, changes in behaviour could have significant effects on the reproduction and interactions of intoxicated animals in their biotope (Osswald et al., 2007). There is a difference in toxicity between the two enantiomers and the route of exposure: (+)-ATX-a being significantly more toxic than (-)-ATX-a (Table 1), while

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the LD<sub>50</sub> is about 60 times smaller through the IP route than through the oral route (Wonnacott and Gallagher, 2006; Metcalf and Codd, 2014).

Finally, a study carried out by administering CB extracts containing ATX-a shows a great difference in sensitivity of various organisms to the toxin: rodent mammals are much less sensitive to ATX-a than avian species (ratio oral LD<sub>90</sub> 2.1 - 5.1), and the goldfish displays a much higher sensitivity than avian and rodent species (ratio oral LD<sub>90</sub> 2.9 - 15.0) (Carmichael and Biggs, 1978). This heterogeneity could explain the fact that certain species may naturally accumulate ATX-a, without effect on their behaviour and fitness and constitute subsequent animal reservoirs of ATX-a, capable of transferring higher amount of this toxin through the food web (see Table 2).

## 7.2. Subchronic and chronic toxicity

Subchronic toxicity by ATX-a force-feeding or added to drinking water has been investigating (Osswald et al., 2007). Due to mortalities observed in groups with higher doses, the oral NOAEL has been estimated at 98 µg.kg<sup>-1</sup>.d<sup>-1</sup> in mice, but the uncertainty on this value remains very high (Fawell et al., 1999). This study provides data on daily chronic oral intoxication with ATX-a in mice (0 to 2.46 mg of ATX-a.kg<sup>-1</sup> during 4 weeks), with no particular symptom in chronic intoxication been retrieved (Fawell et al., 1999). Subcutaneous injection of (+)-ATX-a (0.05 to 0.2 mg/kg) to rats produced tolerance with weekly administration in a food-reinforcement paradigm (Jarema et al., 2008).

Hence, no data are available on a putative subsequent genotoxicity or carcinogenesis of ATX-a. Reprotoxic effects in male mice have been observed with the racemic mixture of ATX-a. An intraperitoneal Lowest Observed Adverse Effect

1 Level (LOAEL) value of 50  $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$  was estimated in male mice after 7 days, on  
2 sperm count (Yavasoglu et al., 2008). Furthermore, maternal toxicity is reported at  
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4 200  $\mu\text{g}\cdot\text{kg}^{-1}$  IP in mice but without generating malformation or mortality in foetuses.  
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6 Conversely, ATX-a effects on foetuses are observed in the hamster: foetal stunting  
7 was observed, without skeletal or soft tissue malformations (Astrachan et al., 1980;  
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9 Fawell et al., 1999; MacPhail et al., 2005; Rogers et al., 2005). In pregnant female  
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11 mice, no sign of toxicity was observed in the offspring. The NOAEL (teratogenicity at  
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13 28 days) is established at 2.46 mg of ATX-a. $\text{kg}^{-1}\cdot\text{d}^{-1}$  (Fawell et al., 1999), as no  
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15 adverse effects were deduced from exposure of pregnant mices by gavage with this  
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17 ATX-a dose (2.46 mg. $\text{kg}^{-1}$  bw per day), nor abnormal foetal development.  
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25 This toxin seems in fact to exert very little effects on the development of other  
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27 vertebrate organisms. Embryos of zebrafish (*Danio rerio*) exposed to 400  $\mu\text{g}\cdot\text{L}^{-1}$  ATX-  
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29 a exhibit variations in heart rate, but these variations are differently modulated  
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31 depending on the stage of development, without being significantly different from the  
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33 control group. At the pec-fin stage (55 h), corresponding to the pectoral fin blade  
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35 development, heart rate was decreased to a mean of 9 % of control embryos, while  
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37 at the protruding-mouth stage (80 h), it was increased to a mean of 12 %. All these  
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39 observations were temporary, and the toxin did not seem to cause chronic effects on  
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41 the development of embryos (Oberemm et al., 1999).  
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47 It can however be noticed that there is a difference between the effects on early  
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49 developmental stages induced by the exposure to pure ATX-a or to cyanobacterial  
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51 extracts of an ATX-a producing strain (Osswald et al., 2009). A challenge with pure  
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53 ATX-a has almost no effect on the development of carp eggs (*Cyprinus carpio*). Only  
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55 a decrease in larval length was observed at the highest concentration (640  $\mu\text{g}\cdot\text{L}^{-1}$ ),  
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57 while all the parameters studied (time to mortality, mortality rate, time to hatching,  
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1 hatching rate, skeletal malformations rate and larval length) were not affected by the  
2 increasing concentrations of CB extracts (Osswald et al., 2009).  
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## 8. Molecular target

### 8.1. Acetylcholine and cholinergic pathways

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ATX-a acts as an agonist of nicotinic acetylcholine receptors expressed in cholinergic synapses, which are widely distributed in the nervous system of vertebrates (Thomas et al., 1993; Swanson et al., 1986; Bertrand and Terry, 2018). Acetylcholine (ACh) is the main neurotransmitter of the peripheral nervous system (PNS) and one of the most important in the central nervous system (CNS). In the CNS, ACh plays a crucial role in a very large number of functions, including cognition or central motor control (Bertrand and Terry 2018). The peripheral cholinergic synapses are found at neuromuscular junctions and in the autonomic nervous system and its effectors (heart, viscera, exocrine and endocrine glands). ACh ensures (i) the transmission of motor pathways which results in the contraction of the skeletal muscles, (ii) the transmission of an excitatory message in ganglia of the parasympathetic and orthosympathic systems and (iii) the post-ganglionic transmission of the parasympathetic pathway. Two types of acetylcholine receptors mediate its action in the postsynaptic cell: ionotropic receptors (called nicotinic receptors, nAChRs) and metabotropic receptors (called muscarinic receptors, denoted mAChRs). After activating these receptors, ACh is hydrolysed by acetylcholinesterase, an enzyme present in the synaptic cleft. This reaction makes it possible to recycle choline in the presynaptic cell (Amenta and Tayebati, 2008). Activation of nAChRs by ACh opens a central pore permeable to cations ( $\text{Na}^+$ ,  $\text{Ca}^{2+}$  and  $\text{K}^+$ ), which promotes the excitation

1 of the postsynaptic cell. From a pharmacological point of view, all nAChRs can be  
2 exogenously activated by the plant alkaloid nicotine. Throughout the discovery of  
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4 new ligands, several pharmacological profiles have been characterized so far, which  
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6 can be linked to their structure. Belonging to the ligand-gated ion channel family,  
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8 nAChRs are expressed as homo- or heteropentamers. To date, 17 subunits have  
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10 been identified in vertebrates:  $\alpha_{1-10}$ ,  $\beta_{1-4}$ ,  $\gamma$ ,  $\delta$ , and  $\epsilon$  (Pedersen, 2019). They can  
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12 organize in not less than 10 different subunit combinations, always including an  $\alpha$   
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14 subunit which is crucial for the ligand binding. There are generally neuronal and  
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16 muscle specific nAChR structures. The muscle nAChR stoichiometry is  $\alpha_1(2)\beta\gamma\delta$  (in  
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18 embryo) or  $\alpha_1(2)\beta\epsilon\delta$  (in adult). It is activated by two ACh molecules which bind to two  
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20 sites located at  $\alpha_1/\epsilon$  and  $\alpha_1/\delta$  interfaces. The activation of nAChRs ends up in  
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22 depolarization and eventually drives the muscle fibre contraction (Martyn et al.,  
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30 2009).

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32 The neuronal nAChRs are heteropentamers with a stoichiometry  $\alpha_x(2)\beta_y(3)$  or  
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34 homopentamers such as  $\alpha_7(5)$ . Heteropentamers and homopentamers carry two and  
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36 five ACh binding sites at the  $\alpha/\beta$  and  $\alpha/\alpha$  interface respectively (Papke, 2014). The  
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38 most widely expressed neuronal nAChRs are  $\alpha_4\beta_2$  and  $\alpha_7$  in the brain and  $\alpha_3\beta_4$  in the  
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40 peripheral nervous system.  
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44 Neuronal and muscle nAChRs can also be expressed in non-neuronal and non-  
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46 muscle tissues, such as vascular endothelium, bones, adrenal medulla, immune  
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48 cells... (Zoli, 2018). The large distribution of nAChRs in the body potentially explains  
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50 the variety of symptoms associated with an inhibition or an over-activation of these  
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52 receptors.  
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## 56 57 58 59 8.2. ATX-a activation of nAChRs 60 61 62 63 64 65

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ATX-a is a potent agonist of nAChRs and to a lesser extent mAChRs (Aronstam and Wiktop, 1981). The agonist activity of ATX-a for mAChRs is weak and requires micromolar concentrations of ATX-a to compete with muscarinic agonists in binding assays (Aronstam and Wiktop, 1981; Aracava et al., 1987), although, at neuromuscular junctions, (+)-ATX-a is a more powerful agonist than acetylcholine for stimulating muscle contraction, due to a greater affinity for nAChRs and a much a slower desensitization kinetics. On neuronal  $\alpha 7$  receptors expressed in *Xenopus* oocytes, (+)-ATX-a exhibits an  $EC_{50}$  of 0.58  $\mu M$ , much lower than acetylcholine  $EC_{50}$  (320  $\mu M$ ) and (-)-nicotine  $EC_{50}$  (24  $\mu M$ ), illustrating the greater potency of the toxin to interact with the receptor (Amar et al., 1993; Thomas et al., 1993). Competitive inhibition assays on membranes of *Torpedo ocellata* electric organs – containing muscle-type nAChRs – evidenced that ATX-a bind at sub-micromolar concentrations to the ACh site in a competitive way towards both ACh and tubocurarine, making the toxin an efficient agonist of the receptor (Aronstam and Wiktop, 1981). Despite its capacity to cross the blood-brain barrier, ATX-a lethal effect can be primarily explained by its potent binding to muscle nAChRs, inducing a sustained depolarizing neuromuscular block (Carmichael et al., 1975).

**Figure 3 near here**

Indeed, in cholinergic synapses, ATX-a is not hydrolyzed by acetylcholinesterase (which naturally degrades ACh) and the nAChRs are therefore overstimulated (Figure 3). This excessive stimulation disrupts the response of the effector organ, which can be a nervous ganglion (autonomous nervous system), a neuron (CNS) or a muscle fibre (sustained contraction) (Spivak et al., 1980; Wonnacott et al., 1992;

1 Thomas et al., 1993). At the molecular level, ATX-a very effectively activates the  
2 muscle nAChR  $\alpha_1(2)\beta\epsilon\delta$ . Through its binding to these nicotinic receptors, ATX-a  
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4 causes the opening of their cationic pore, generating an influx of  $\text{Na}^+/\text{Ca}^{2+}$  ions into  
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6 the postsynaptic cell and an efflux of  $\text{K}^+$  ions, which leads to membrane  
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8 depolarization. Prolonged exposure to ATX-a causes desensitization of nAChRs, and  
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10 blockade of neuromuscular transmission (Spivak et al., 1980). HTX-a acts in a similar  
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12 way as ATX-a: their affinities for nAChRs are comparable (Wonnacott et al., 1992).  
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15 In addition to these blocking effects of neuromuscular transmission and taking into  
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17 account the fact that nAChRs are present in autonomic nervous system, their  
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19 activation at interneurons of parasympathetic ganglia induces the release of ACh  
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21 which stimulates post-synaptic muscarinic receptors on organs. Consequently, ATX-  
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23 a affects the cardiovascular system: it causes a decrease in heart rate, an increase in  
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25 blood pressure, and disruptions of gas exchanges ( $\text{pO}_2$  and  $\text{pCO}_2$ ), which leads to  
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27 hypoxia and respiratory arrest, as well as acidosis observed in animal deaths  
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29 (Adeymo and Sire'n, 1992). In the adrenergic system, ATX-a acts on post-ganglionic  
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31 and adrenal nAChRs and stimulates the secretion of catecholamines (dopamine,  
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33 adrenaline and noradrenaline) which display multiple effects in the CNS and PNS  
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35 (cardiac function, metabolic functions). ATX-a crosses the blood-brain barrier and  
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37 exerts its nAChR agonist activity (Figure 3), but less effectively than on  
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39 neuromuscular junctions (Carmichael et al., 1977; Stolerman et al., 1992). The most  
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41 deleterious effects of ATX-a are therefore due to excessive stimulation of the skeletal  
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43 muscles of the respiratory tract, which can lead to respiratory arrest (Spivak et al.,  
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45 1980; Aronstam and Witkop, 1981; Swanson et al., 1986).  
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## 9. Regulatory thresholds

1 Numerous animal intoxications involving CB-producing ATX-a have been published  
2 and show that it is a harmful toxin well disseminated in the environment. The  
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4 symptoms observed experimentally or on field emphasize its neurotoxic potency, but  
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6 also its cardiovascular effects in acute exposure. Despite the risk, ATX-a is scarcely  
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8 regulated. Guideline values have been proposed locally in some countries: 5  $\mu\text{g}\cdot\text{g}^{-1}$   
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10 in fish flesh in California; 40 and 100  $\mu\text{g L}^{-1}$  in drinking water for livestock and dogs,  
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12 respectively, in California; 3.7  $\mu\text{g}\cdot\text{L}^{-1}$  in drinking water in Quebec (used as a  
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14 temporary acceptable maximum dose); 6  $\mu\text{g}\cdot\text{L}^{-1}$  and 2  $\mu\text{g}\cdot\text{L}^{-1}$  in drinking water for  
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16 ATX-a and HTX-a respectively, in New Zealand (used as the provisional maximum  
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18 permissible dose); 20  $\mu\text{g}\cdot\text{L}^{-1}$  and 3  $\mu\text{g}\cdot\text{L}^{-1}$  in drinking water in Ohio and Oregon,  
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20 respectively (Farrer et al., 2015). No threshold currently exists in Europe for ATX-a or  
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22 HTX-a. Recently, ATX-a producers were also evidenced for the first time in Australia,  
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24 highlighting the need for more frequent investigation of the distributions of toxin-  
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26 producing CB in this region (John et al., 2019). Given its harmful character, its  
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28 growing frequency in CB blooms and its involvement in animal poisoning, regulatory  
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30 ATX-a thresholds aim at being more carefully investigated.  
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## 42 **10. Concluding remarks**

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44 Studies on ATX-a and its structural analogues have resulted in a relatively  
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46 satisfactory characterization of the ecological causes and circumstances under which  
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48 this toxin is produced by CB, present in the water and the food web. These factors, in  
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50 particular of anthropogenic origin, which favour CB proliferation, comprise  
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52 eutrophication and perturbations of river hydric regime. The acute toxicity of ATX-a  
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54 has been the subject of fairly old work, and its neurotoxic tropism has been  
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56 evidenced, even if the toxicokinetic data are still quite fragmented. ATX-a, by its  
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chemical structure and its physicochemical properties, seems to be able to cross most biological membranes – including the gastro-intestinal tract, the blood-brain barrier and the placenta – and quickly reaches its molecular target. After causing deleterious effects in the nervous system, it is rapidly excreted, and no sequels are evidenced at sublethal doses. The toxin binds with high affinity to nAChR, which is considered so far as its sole target. This ionotropic receptor is expressed in the central and peripheral cholinergic pathways. When bound, ATX-a causes nAChR persistent opening, compromising the communication between neuronal and postsynaptic cells, at the level of the skeletal muscle, cardiovascular and CNS. The symptoms observed in animals on field or through experimental exposure can therefore be explained by this ATX-a/nAChR interaction. There is no human epidemiological data sufficiently reliable to link human intoxication to exposure to this toxin, with the exception of one case recently described in the south of France. We can anticipate that a human acute intoxication linked to ATX-a would be managed through symptomatic treatments. The chronic toxicity of ATX-a remains however not characterized, and a long-lasting exposure is still possible, due to the recurrent CB bloom occurrence. Finally, the differential sensitivity to ATX-a of various animal species still specifically deserves to be the subject of future studies. Indeed, some animals seem to resist to this toxin better than others and the toxicokinetic or toxicodynamic determinants of this "resistance" are not yet known. Regulatory thresholds for water contamination should be proposed to consider and prevent possible human intoxication.

#### **Declaration of competing interest**

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6 The authors declare no conflicts of interest.

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## Figure captions

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3 Figure 1 : ATX-a producing cyanobacteria. (A) *Phormidium* sp bloom in the Cher  
4 river, France (© Jean-François Humbert). (B) *Phormidium* sp biofilm (© Jean-  
5 François Humbert). (C) *Phormidium favosum* from the Tarn river, France, observed  
6 with scanning electron microscopy. Note the diatoms at the centre (© Isidora  
7 Echenique-Subiabre)  
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19 Figure 2: Structures of ATX-a and related molecules.  
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25 Figure 3: Cellular and molecular mode of action of ATX-a. (A) ATX-a binds and  
26 opens nAChRs, but unlike their natural ligands – ACh – the toxin does not get  
27 hydrolyzed by resident acetylcholinesterases. (B) ATX-a targets cholinergic synapses  
28 where nAChRs are expressed, in both CNS and PNS. (C) Through its rapid  
29 absorption, ATX-a exerts deleterious effects on the brain, the cardiovascular system,  
30 the respiratory tract and the skeletal muscles.  
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Table 1: Main episodes of ATX-a intoxication in the wild. DAD, Diode Array Detector, DW Dry Weight; ELISA, Enzyme Linked ImmunoSorbent Assay; ESI, Electrospray Ionisation; FW, Fresh Weight; GC/ECD, GC Gas Chromatography with Electron Capture Detector; MS, Mass Spectrometry; HPLC, High-Performance Liquid Chromatography; MALDI-TOF, Matrix Assisted Laser Desorption Ionization - Time Of Flight; NI, not indicated; PDA, Photometric Diode Array.

Year, Country and Location	Intoxication and mortalities episode	Method of detection	Matrix	Congener and maximum concentration	Cyanobacteria species (benthic/planktonic)	Reference
1981, USA, Black Lake	11 cows died 2 dogs died	GC/ECD	Water sample	2.5 g.kg <sup>-1</sup> (DW) of ATX-a	<i>Anabaena flos-aquae</i> (planktonic)	Stevens and Krieger, 1988
1985, Canada, Steele Lake	1000 bats died, 24 mallards and American wigeons died	GC-MS	Green slime from the carcasses	ATX-a	<i>Anabaena flos-aquae</i> (planktonic)	Pybus et al., 1986
1985-1986, Finland, Lakes Sääskjärvi and Säyhteenjärvi	5 cows died	HPLC, GC-MS	Water sample	3.7 g.kg <sup>-1</sup> (DW) of ATX-a	<i>Anabaena</i> spp. (Planktonic)	Sivonen et al., 1990
1987, Canada, Alberta	16 cows died	GC-MS	NI	ATX-a	<i>Anabaena flos-aquae</i> (Planktonic)	Smith and Lewis, 1987
1990-1991, Scotland, Loch Insh	4 dogs died and one intoxicated	HPLC-DAD, GC-MS	Water sample	ATX-a	<i>Oscillatoria</i> spp. (benthic)	Edwards et al., 1992
			Dog stomach content	ATX-a		
1992-1993-1994, Ireland, Caragh Lake	6 dogs died	HPLC-FL, GC-MS	Water sample	444 µg.L <sup>-1</sup> (FW) of ATX-a	<i>Oscillatoria</i> spp. (benthic)	James et al., 1997
1998-1999-2000, New Zealand, Waikanae and Mataura Rivee	12 dogs died	HPLC	Cyanobacterial mat sample	ATX-a	<i>Oscillatoria</i> spp. (benthic)	Hamill, 2001
2001, Kenya, Lake Bogoria	Mortalities of Lesser Flamingos	HPLC-PDA, MALDI-TOF	Cyanobacterial mat sample	18 mg.kg <sup>-1</sup> (DW) of ATX-a	<i>Phormidium terebriformis</i> (benthic),	Krienitz et al., 2003

			Flamingo stomach content	4.349 mg.kg <sup>-1</sup> (FW) of ATX-a	<i>Oscillatoria willei</i> (benthic), <i>Spirulina subsalsa</i> (benthic) and <i>Synechococcus bigranulatus</i> (benthic)	
			Flamingo intestine	0.762 mg.kg <sup>-1</sup> (FW) of ATX-a		
			Flamingo pellet	0.245 mg.kg <sup>-1</sup> (FW) of ATX-a		
2001-2002 Kenya, Lakes Bogoria and Nakuru	Mortalities of Lesser Flamingos	HPLC-PDA, MALDI-TOF	Water sample	223 mg.kg <sup>-1</sup> (DW) of ATX-a	<i>Arthrospira fusiformis</i> (planktonic)	Ballot et al., 2004
2003, France, La Loue River	2 dogs died	ESI-MS/MS	Cyanobacterial mat sample	8 g.kg <sup>-1</sup> (DW) of ATX-a	<i>Phormidium favosum</i> (benthic)	Gugger et al., 2005
			Dog liver extracts	0.6 g.kg <sup>-1</sup> (DW) of ATX-a		
2005, Kenya, Lakes Bogoria and Nakuru	Mortalities of Lesser Flamingos	HPLC-PDA, MALDI-TOF	Feathers	800 µg.kg <sup>-1</sup> (DW) of ATX-a	n.r.	Metcalf et al., 2006
2005, New Zealand, Hutt River	5 dogs died	LC-MS	Cyanobacterial mat sample	27 µg.kg <sup>-1</sup> (FW) of ATX-a	<i>Phormidium autumnale</i> (benthic)	Wood et al., 2007
				4400 µg.kg <sup>-1</sup> (FW) of HTX-a		
2006, Canada, pond in Ontario	3 dogs died	LC-MS/MS/MS	Dog stomach content	10 µg.kg <sup>-1</sup> (?) of ATX-a	<i>Planktothrix</i> spp. (benthic)	Puschner et al., 2008
2011, Netherlands, Lake IJmeer	3 dogs died and 1 dog intoxicated	LC-MS/MS	Cyanobacterial mat sample	272 µg.g <sup>-1</sup> (DW) of ATX-a	<i>Phormidium</i> sp. (benthic)	Faassen et al., 2012
			Dog stomach content	9.5 µg.g <sup>-1</sup> (DW) of ATX-a		
2014, New Zealand, pond near Kaikoura	1 dog died	LC-MS/MS	Environmental sample	25 µg.L <sup>-1</sup> (FW) of ATX-a	<i>Phormidium autumnale</i> (benthic)	Wood et al., 2016
				2118 µg.L <sup>-1</sup> (FW) of dhATX-a		
				Dog stomach content		
4437 µg.kg <sup>-1</sup> (FW) of dhATX-a						
2015, Iran, Shoormast Lake	Ducks died	LC-MS	Duck stomach content	ATX-a	<i>Nostoc</i> sp. (benthic), <i>Anabaena</i> sp. (planktonic) and <i>Nodularia</i> sp (planktonic)	Nowruzi et al., 2018
2016, Russia, Kazanka River	1000 gibel carps died	ELISA kit	Water sample	0.294 µg.L <sup>-1</sup> (FW) of ATX-a	<i>Planktolyngbya limnetica</i> (planktonic) and	Stepanova et al., 2018
			Fish muscles	5.211 µg.kg <sup>-1</sup> (FW) of ATX-a		
			Duck muscles	1.559 µg.kg <sup>-1</sup> (FW) of ATX-a		



	10 ducks died		Duck Liver	5.193 $\mu\text{g}\cdot\text{kg}^{-1}$ (FW) of ATX-a	<i>Aphanizomenon flos-aquae</i> (planktonic)	
2017, Germany, Lake Tegel	3 dogs died	LC-MS/MS	Water sample	1 $\mu\text{g}\cdot\text{L}^{-1}$ (FW) of ATX-a	<i>Tychonema</i> sp. (planktonic)	Fastner et al., 2018
			Sand sample	1 $\mu\text{g}\cdot\text{L}^{-1}$ (FW) of ATX-a		
			Cyanobacterial mat sample	1870 $\mu\text{g}\cdot\text{L}^{-1}$ (FW) of ATX-a		
			Dog stomach content	8870 $\mu\text{g}\cdot\text{L}^{-1}$ (FW) of ATX-a		
2017, France, La Loire and Le Cher rivers	12 dogs died			ATX-a	<i>Phormidium</i> sp. (benthic)	Boisseleau et al., 2018

Table 2: Acute *in vivo* toxicity of ATX-a in vertebrates

Toxin used or CB extract	Route of administration	Species	LD <sub>50</sub> (mg.kg <sup>-1</sup> )	LD <sub>90</sub> (mg.kg <sup>-1</sup> )	References
(+)-ATX-a	Oral	mouse	16.2		Stevens and Krieger, 1991
(+)-ATX-a		medaka fish	5.75		Colas et al., 2020
(±)-ATX-a		medaka fish	11.5		Colas et al., 2020
(+)-ATX-a	IP	mouse	0.26		Stevens and Krieger, 1991
(+)-ATX-a	IV	mouse	0.39		Valentine et al., 1991
(±)-ATX-a		mouse	0.91		Valentine et al., 1991
(+)-ATX-a		rat	0.08		Adeyemo and Sirén, 1992
(±)-ATX-a		rat	0.4		Adeyemo and Sirén, 1992
<i>Anabena</i> floS- aquae NRC-44-1	Oral	mouse rat pheasant		1800 1500 850	Carmichael and Biggs, 1978

		duck		350	
		gold fish		120	

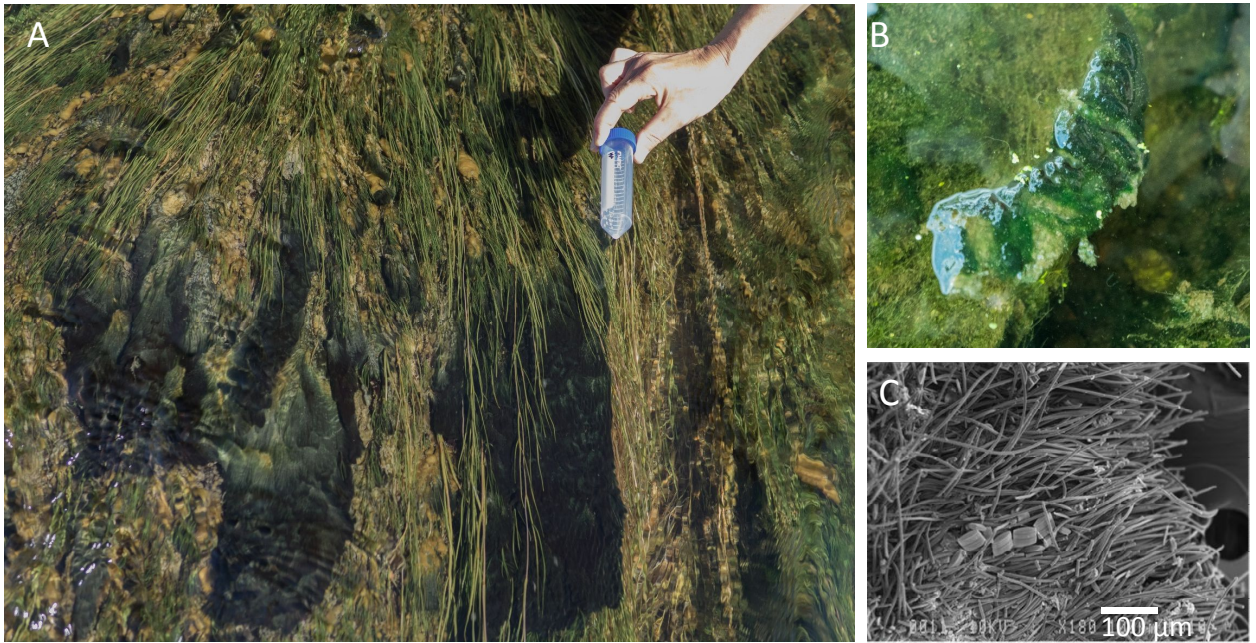


Figure 1

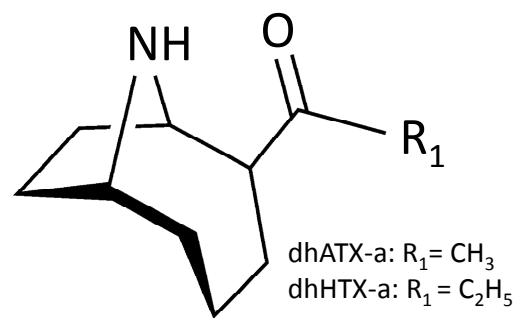
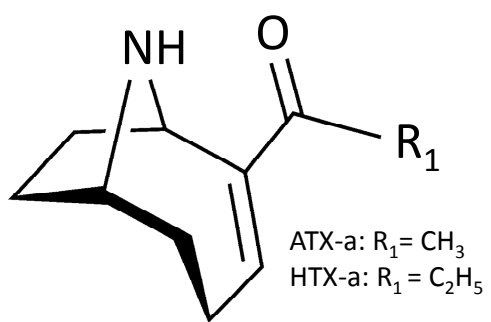
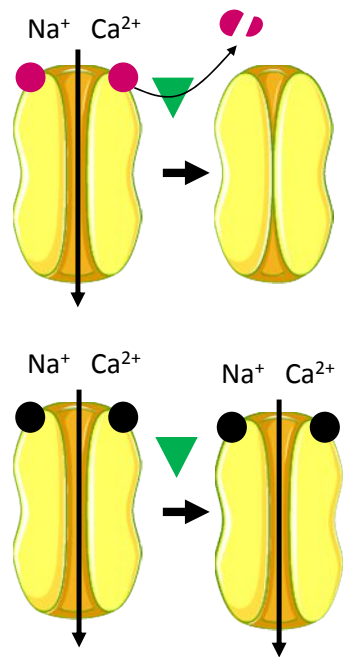


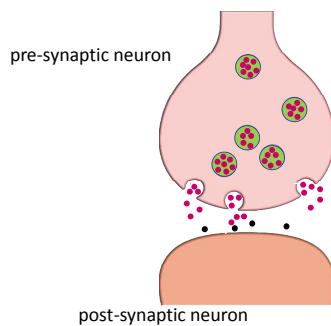
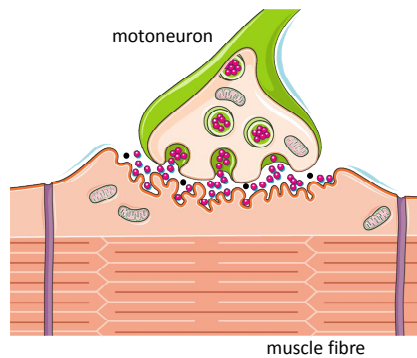
Figure 2

A



- Acetylcholine
- ATX-a
- ▲ Acetylcholinesterase

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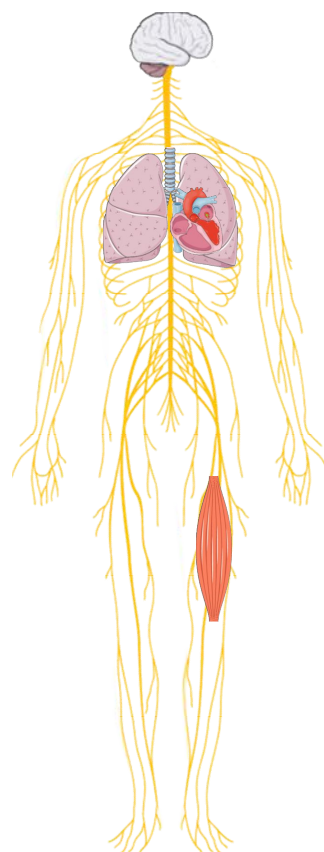


Figure 3

**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: