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All the authors declare no conflict of interest.

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ABSTRACT

Atmospheric CO_2 concentrations increased significantly over the last century and continuing increases are expected to have significant effects on current ecosystems. This study evaluated the behavioural and physiological (hormone status, muscle structure) effects of prolonged CO_2 exposure in young female Wistar rats exposed at 700 ppm of CO_2 during 6 h a day for 15 days.

Prolonged CO_2 exposure, though not continuous, produced significant disturbances in behaviour with an increase in drinking, grooming and resting, and a reduction in rearing, jumping-play and locomotor activity. Furthermore, CO_2 exposure was accompanied by increased plasma levels of corticosterone, suggesting that prolonged exposure to CO_2 was stressful. The muscular structure can also be modified also when respiratory working conditions change. The expression of myosin heavy chain was significantly affected in the diaphragm and oral respiratory muscles: *Masseter Superficialis* and *Anterior Digastric*. Modified behaviour and hormonal changes both appear to be at the origin of the observed muscular adaptation.

Keywords: behaviour, hormones, CO_2 exposure, rats, respiratory muscle structure, stress

Introduction

Atmospheric carbon dioxide (CO_2) concentrations began increasing with the advent of the industrial revolution and they continue to increase at a rate of approximately 4 parts per million by volume (ppm) annually (20). Over the last two decades, the average CO_2 concentration in the Earth's atmosphere showed a steady rise from 357 ppm in 1992 to 389 ppm in 2011 (53). In 2013, the daily average concentration of carbon dioxide (CO_2) in the atmosphere surpassed 400 ppm (12). Moreover, the mean ambient urban CO_2 concentration (23, 24) is around 400 ppm. At the current rate of increase, CO_2 levels are expected to increase to 750 ppm at the end of this century (20). The increase is expected to have significant effects on physiological changes in all living organisms. Numerous studies have been conducted to determine the effects of elevated CO_2 on plants and their associated communities (behavioural and/or physiological changes in herbivores), but few directly on mammals. On the other hand, work concerning the effects of CO_2 on mammals has used very high doses of CO_2 (20,000 to 60,000 ppm), *i.e* levels not commonly found in the environment or in the future probable atmosphere. These high doses of CO_2 caused different modifications: hypercapnia, sedation, increased respiratory behaviour (22, 33, 41). Chronic exposure to CO_2 (50,000 ppm), at 6 time points in a 24-h period, increased plasma level of corticosterone in rats (2). These studies showed that an increased CO_2 level in the atmosphere was perceived as a stressful situation by the experimental animals.

However, in addition to CO_2 exposure, the individual's environment also seems to have an impact. Indeed, Clougherty et al. revealed a link between traffic-related air pollution and asthma solely among urban children exposed to familial violence,

suggesting that air pollution was a stressor amplified in the already stressed patient (10). Moreover, Bhat et al. showed that the shape of a rat's housing box could counteract plasma cortisol elevation and oxidative stress caused by chronic restraint. This later study showed that the effect of a stressing agent was fully dependent on the state of the individual and on how he interacted with his environment (4).

Indeed, stress was showed to induce changes of different physiological variables, such as plasma hormone levels and in particular glucocorticoid and thyroid hormones (18, 26, 27, 52). Furthermore, two studies (3, 25) showed that thyroid and glucocorticoid hormones were implicated in the modification of the myosin heavy chain (MHC) isoform expression. Other studies demonstrated that the administration of corticoids affected the diaphragm structure and function (11, 54, 58). This was accompanied by type IIB fibre atrophy in the diaphragm as well as in the peripheral skeletal muscles (43).

The administration of CO_2 by the re-breathing technique produced an increase in the breathing activity amplitude, and the respiratory frequency increased due to a decrease in both the inspiratory and expiratory durations (40). These modifications changed the working conditions of the respiratory muscles, implying a phenotypical adaptation of the muscles (44, 47, 50). These phenotypic modifications to the new environmental requirements generally involved muscle structure, in particular MHC isoform composition (6, 17, 21, 31).

Many studies on CO_2 were carried out in male animals while other results suggested that female rats were more reactive to stress than males (37, 56). Thus for our studies we chose to work on young female rats. Moreover, Cantillon and

Bradford reported that rat gender did not affect upper airway muscle contractile properties (9).

Although many physiological and behavioural effects were described using high doses of CO_2, little is known about the effects of relatively low doses, thus the aim of this present study was to investigate the behaviour, hormonal status (thyroid and glucocorticoid hormones) and respiratory muscle structure of rats chronically exposed to an eventual future condition of atmospheric CO_2 (700 ppm). A non-respiratory muscle related to locomotion, the soleus, was also analysed to determine any peripheral effects of elevated CO_2 exposure.

Materials and methods

Animals and breeding procedures

All experiments were carried out in accordance to the *Guide for the Care and Use of Laboratory Animals* published by the National Research Council (36), the recommendations edited by the European Union (Directive 2010/63/EU) (13), and those of the *Centre National de la Recherche Scientifique* (CNRS, France).

Twenty four female Wistar rats aged 6 weeks, weighing 152 ± 3g, were purchased from a commercial breeder (IFFA-CREDO, France). On the arrival at the laboratory, the animals were housed in an air-conditioned room (temperature 30 ± 1°C, relative humidity 50 ± 2 %) with the lights on from 8:00 pm to 8:00 am (inversion of the natural light cycle to adapt to the natural life conditions of rats which are nocturnal animals). The animals were housed individually in 60x38x20-cm Plexiglas boxes with a metal top and sawdust as bedding. Pellet food and tap water were continuously available. After 1 week of acclimatization, 12 females

were randomly assigned to the CO_2 exposure group, and the other 12 females to the control condition. The animals were kept all the time under filtered air except for the periods of CO_2 exposure in the experimental group. The experiments were performed at the same time of the day and under identical conditions, to insure that all groups were comparable.

Exposure apparatus and experimental procedure

The apparatus included a device for the production of CO_2, a control system for a continuous delivery of CO_2 (with concentration monitoring and recording), and four stainless-steel exposure chambers (100 x 180 x 150 cm) with a hatch glass in the front door. The chambers were located in an air-conditioned room and were equipped with racks which could hold up to 6 Plexiglas housing boxes (60 x 38 x 20 cm).

The control females were treated with charcoal-filtered (CF) air (453.2 ± 13.4 ppm CO_2). All experimental animals were exposed during 15 days to CF air plus 250 ppm CO_2 (685.9 ± 30.2 ppm). These chronic treatments were performed for 6 hours a day during the period of behavioural activity (12:00 AM to 06:00 PM).

Behavioural observations

The behaviour of the rats in their housing boxes was observed on the first day of exposure to CO_2 and after 15 days of exposure. The rats were exposed to CO_2 from 12:00 AM and 06:00 PM and all the observations were carried out during the period of stable gas levels i.e between 03:00 and 06:00 pm.

The behaviour was observed independently and by two experienced and blinded-to-condition observers. The data were recorded using a keyboard event recorder system feeding to a computer for analysis. Separate scores were obtained for each individual. Six types of behaviours displayed by each animal were recorded during a 5 min test with measures being taken at 1s intervals. The six behavioural scores were based on the ethological profile of rodents: inactivity (lying flat or standing still, with eyes open or closed and total absence of movements), jumping-play (jumping up vertically to a wall or to the metal top), rearing (standing on hind legs), grooming (wiping, licking, combing or scratching of any part of the body), drinking and eating.

Physiological analysis

After a 15-day exposure period, the animals were anaesthetized (sodium pentobarbital, i.p., 40mg/kg) and blood samples taken to analyse their physiological state. This was carried out in the neighbourhood around 09:00 am for all animals to minimise diurnal hormonal variations.

Immediately after the anaesthesia (30 s), intra-cardiac blood samplings (1 ml) were performed for corticosterone, thyroxine and triiodothyronine measurements (the duration of blood taking, starting from the departure of the animal from the home box, never exceeded 3 min).

The blood was collected into sterile heparinised syringes fitted with a 26-G needle. For the corticosterone assay, the plasma was immediately separated from the red blood cells by centrifugation at 4°C (15 min at 3,000 rpm) then the extracts were stored at -18°C until the time of the assay. The corticosterone concentration

was measured without an extraction procedure, using a commercially available EIA kit (Assay Designs Inc., USA). The concentration of corticosterone in the plasma samples was calculated from a standard curve and expressed in ng/ml. The intra- and inter-assay coefficients of variation were under 8.4 and 13.1 %, respectively.

Free thyroxine (FT4) and triiodothyronine (FT3) were assayed using commercial RIA kits according to the manufacturer's guidelines (Immunotech SA, France). The concentrations of FT4 and FT3 in the plasma samples were calculated from standard curves and expressed in pg/ml. The intra- and inter-assay coefficients of variation were respectively under 6.7 and 6.5 % for FT4 and under 6.4 and 5.5 % for FT3.

Muscle sampling and myosin extraction

Samples from one locomotor and four respiratory muscles were removed from the animals: the *Soleus* (S, plantarflexion of the foot); *Masseter Superficialis* (MS, propulsive and elevator mandibular muscle, i.e. mouth closing) and *Anterior Digastric* (AD, depressor mandibular muscle, i.e. mouth opening), antagonist muscles related to mouth movements and oral breathing (49, 57); *Levator Nasolabialis* (LN, active sniffing muscle) related to nasal breathing (48); and the Diaphragm (Dia). After dissection, the muscle samples were immediately frozen in liquid nitrogen for protein electrophoretic analyses. Myosin was crudely extracted in a high ionic strength buffer, as described by d'Albis et al. (1).

Electrophoretic analysis of MHC and quantification

The electrophoresis was performed according to the method of Talmadge and Roy (51). Mini-gels were used in the Bio-Rad Mini-protean II Dual Slab Cell. The electrophoresis was performed in a refrigerated room, at a temperature of 6°C for the whole run. To separate all the Myosin Heavy Chains, the duration of the run was of 28 h. The gels were stained with Coomassie blue R-250. The relative amounts of the different MHC were measured using an integration densitometer Bio-Rad GS-800 and analysed with the Quantity one 4.2.1 Program. Only bands representing more than 1% of total MHC were taken into account.

Statistical methods

All results were expressed as mean ± SEM. The behavioural and physiological data were normally distributed; Student's *t*-test was used to establish the inter-group comparison. Differences were considered significant at $p < 0.05$.

Results

Behaviour

The analysis of the different behaviours is presented in Figs. 1-3. The results in Figure 1 run from the end of the first week of acclimatisation to the experimental setup. It is clear that there were no differences between the two experimental groups. Fig. 2 shows the effect on the behaviour after the first day of exposure to CO_2. During this first day of exposure, there was a significant decrease in rearing ($t = 15.96$, $p = 0.0003$), in association with a significant increase in inactivity ($t = 6.38$, $p = 0.01$), grooming ($t = 4.93$, $p = 0.03$) and drinking ($t = 4.07$, $p = 0.05$).

After 15 days of exposure (Fig. 3), these significant differences were accentuated: rearing ($t = 20.64$, $p < 0.0001$), inactivity ($t = 7.70$, $p = 0.005$), grooming ($t = 9.26$, $p = 0.004$), and drinking ($t = 6.83$, $p = 0.01$). Furthermore, other behaviours were observed to decrease after this long period of exposure: jumping-activity ($t = 5.45$, $p = 0.02$), and locomotor activity ($t = 4.06$, $p = 0.05$).

CO_2 exposure produced a significant increase in the daily amount of water consumed by each female after the first day: mean for the 14 days, 32 ± 1 ml/day *vs* 27 ± 1 ml/day for control females ($t = 2.85$, $p = 0.05$). The amount of food consumed every day by each animal was similar between the two groups of females: mean for the 15 days, 21 ± 4 g / day for control and 20 ± 2 g / day for ozone group ($t = 0.91$, $p = 0.35$).

Physiological analyses

The plasma levels of corticosterone were increased significantly by CO_2 exposure (Fig. 4): (240 ng/ml in CO2 *vs.* 87 ng/ml in Controls, $t = 5.2$, $p = 0.01$).

FT3 and FT4 plasma levels were unchanged under CO_2 *versus* control group (respectively 3.19 pg/ml *vs.* 3.18 pg/ml, $t = 0.07$, $p = 0.78$; 16.92 pg/ml *vs.* 15.67 pg/ml, $t = 1.9$, $p = 0.19$, Fig. 4).

Muscle analyses

The body mass of the rats (185.8 ± 2 g CO_2 group, 194.5 ± 3,6 g control; $t = 4.4$ $p = 0.06$) as well as the relative mass of the five studied muscles was not significantly different between control and CO_2 groups (for the relative mass of the five muscles; $0.1 < t < 2.8$, $0.1 < p < 0.8$). The order of increasing electrophoretic

mobility of adult MHC isoforms was the following: fast 2A, fast 2X, fast 2B and slow 1 type (Fig. 5). Proportions of MHC isoforms were expressed as a relative percentage of the total amount of MHC present in the muscles studied (Table 1).

In the five muscles under control conditions, the 3 fast MHC isoforms could be observed, MHC 2A, 2X and 2B, except for LN in which the MHC 2A isoform was not detected and for S in which only MHC 2A was detected (Table 1). In relative expression, MHC 2B was the most abundant isoform in the AD and LN (44.7 %, 71.2 % respectively), whereas in the MS, MHC 2X was the predominant isoform (47.8 %). MHC 2A had the lowest expression level in S, AD and MS (15.8 %, 22.7 %, 23.9 % respectively). The slow type, MHC 1, was present only in S and Dia (84.2 %, 26.7 % respectively). The distribution between the different MHC isoforms was equivalent in Dia. In the soleus muscle, the slow isoform MHC 1 and only one fast isoform MHC 2A were detected. In relative expression, MHC 1 was the most abundant isoform (84.2 %).

During CO_2 exposure, the relative amounts of MHC protein isoforms were significantly affected in four of the five studied muscles. Indeed, the relative proportion of MHC 2B and MHC 1 significantly decreased to the detriment of MHC 2A in Dia under CO_2 exposure *versus* control. In addition, the relative proportion of MHC 2X increased in AD and decreased in MS. In the soleus muscle, the relative amounts of MHC protein isoforms were also significantly altered with an increase in the relative proportion of MHC 1 and a decrease of MHC 2A.

Discussion

These results specifically show that in sexually immature female rats, during prolonged CO_2 exposure (700 ppm, for 6 hours a day, during 15 days), there was an increase in drinking, grooming and inactivity behaviours, with a decrease in physical activity behaviours (jumping, rearing and locomotor activity).

Previous work concerning the effects of CO_2 on mammals generally used very high doses of CO_2 (20,000 to 60,000 ppm), ie levels not commonly found in the environment or in the future probable atmosphere. Wade et al. used experimental conditions close to the present study, in that they studied rat growth, body composition, and renal function during 30 days of increased ambient CO_2 (3,000, 7,000 and 20,000 ppm) (55).

The increase in drinking could be explained by a specific effect of this exposure to CO_2 which was not continuous. The regulation of breathing relies upon a chemical feedback concerning the levels of CO_2 and O_2. The feedback for CO_2 involves multiple sites including the carotid and receptors in the brain (15, 28). Small increases in CO_2 produce large increases in breathing. For instance, in awake humans at rest, a 1 mmHg increase in $PaCO_2$ leads to increased ventilation by ~20 - 30% (15). This could easily have increased the respiratory evaporative water loss (46) and thus produced dehydration which stimulated drinking. Wade et al. showed a reduced water turnover in male rats exposed to 3,000, 7,000 and 20,000 ppm (by increased urine output or by reduced water intake) during 30 days (55). They also showed that there were no negative water balances in spite of reduced water turnover.

The amount of food consumed every day by each animal was similar between the two groups of females. Wade et al. showed a slight decrease in food intake, yet the body weight, lean body mass and fat content remained relatively similar (55).

After a chronic exposure to CO_2 there was a significant decrease in rearing, in association with a significant increase in inactivity behaviour, grooming, jumping-activity, and locomotor activity. The observed decrease of rearing can be related to the procedure of behavioural observation in a familiar environment (the housing box) and the particular CO_2 exposure. The reduced physical performance during CO_2 exposure can be linked with another pollutant, O_3 (32), which is supposed to be part of the mechanisms preventing sensory irritation (29).

At CO_2 exposure levels of 10,000-40,000 ppm, the observed deleterious effects on the central nervous system are considered low to mild, and consist in headaches, a reduction of stereoacuity and a decrease in the ability to detect motion (19). A number of studies in humans suggested that CO_2 exposures in the range of 15,000-40,000 ppm did not impair neurobehavioral performances but led to clinical observations of poor attention, erratic behavior, confusion, motor skill impairment and moderate increases in anxiety, apathy, uncooperativeness, desire to leave, and sexual desire (35).

These behavioural changes suggest that chronic exposure to CO_2 could be a stressful situation. Furthermore, the CO_2 exposed group presented increased plasma levels of corticosterone which could be in relation with anxiety behaviour manifested by these females. Chronic exposition to CO_2 (50,000 ppm) increased the plasma level of corticosterone in rats as shown by Altholtz and colleagues (2). Wade et al. showed, in the male rat, an increase of plasma corticosterone only at

20,000 ppm and not at 3,000 and 7,000 ppm (55). To our knowledge, this is the first time that an increased plasma corticosterone level has been shown following chronic exposure to low levels of CO_2.

Chronic episodic hypercapnic hypoxia alters upper airway muscle structure and function in the rat. With respect to maximum velocity of shortening, the type 2B fibre is thought to have the highest velocity, followed by 2X > 2A > 1 (6). Our results have confirmed that the four muscles studied (Dia, LN, AD and MS), which predominantly expressed the type 2 MHC isoforms, were fast-twitch muscles. Among these fast muscles, the LN muscle, containing mainly type 2B (71%), was faster than the AD muscle, containing 45% type 2B, 33% type 2X and 23% type 2A, which in turn was faster than the MS muscle, containing mainly 2X type (48%). Lastly, Dia which contains about the same proportion of the four isoforms is the slowest muscle.

Redline and Strohl showed that the electromyographic activities of the upper airway and thoracic muscles increased at similar rates with an increase in hypercapnia (39). In the CO_2 exposition group, the modifications of MHC distribution in AD and MS could be explained by a modification of the electromyographic activity in these upper air way muscles. Geniohyoid type 1 fibers were reduced and type 2B fibers increased, whereas the sternohyoid muscle had an increase in type 1 and 2A fibers and a decrease in type 2B fibers (34). The primary explanation for these modifications is the working conditions of the muscles, whereas our experimental results bring other possible factors to light such as the hormonal factors or the intracellular pH. CO2, by diffusing across the

lipid bilayer, can have an effect on intracellular pH. A low pH can alter neuronal function and synaptic transmission (5, 41).

Various hormones are known to cause changes in the expression of MHC isoforms. Thyroid hormones could be an important factor implicated in the muscle structure changes observed after a stressful situation (1, 16) as they are very important in the normal development of vertebrate skeletal muscles (16). All members of the myosin heavy chain multigene family respond to T3. However, the mode of response is not intrinsic to any myosin heavy chain gene, but is determined in a highly muscle type-specific manner (25, 30). Caiozzo et al. showed that combined effects of hyperthyroidism (T3) and a stressful procedure like hindlimb suspension (HS) rapidly converted the soleus into a fast muscle, producing large increases in the relative contents of the fast type 2X and 2B MHC isoforms which were primarily expressed in several populations of hybrid fibres (e.g., types 1/2A/2X, 1/2X/2B, 1/2A/2X/2B) (7). Finally, T3 + HS produced unique populations of hybrid fibers that did not adhere to the 1/2A/2X/2B sequential scheme of MHC plasticity. In a previous publication we showed that under chronic O_3 breathing, the serum level of FT3 was increased, which could have explained the appearance of hybrid fibres responsible for the modification of MHC profile in Dia, AD and MS (32). In contrast to ozone exposure, as shown here, chronic exposure to CO_2 did not modify serum levels of FT3. This could be explained by two mechanisms acting in opposite directions; the glucocorticoid release increased, while the uncontrollable stress inhibited the secretion of thyroid hormones (26, 38).

Chronic exposition to CO_2 increased the plasma level of corticosterone in rats. Falduto et al. showed that glucocorticoid treatment caused an increase in myosin heavy chain 2A expression (14). Glucocorticoids significantly decreased the relative expression of myosin heavy chain isoform MHC 2B in the Dia (38). The level of corticosterone could be one among other hormonal factors acting on muscle structure under CO_2.

In a previous study, Martrette et al. showed that a controllable stressful situation induced a marked increase in the relative expression of MHC 2B in MS and AD muscles, making the studied muscles faster in contraction (31). The authors concluded that in a controllable stress situation, there were probably several factors acting upon muscle MHC distribution. First, behavioural changes could be one of these factors (21). Thus, when the working conditions are changed, marked transitions in the myosin content occur in rat fast- and slow-twitch muscles (50). These modifications generally adapt the muscle to the new environmental requirements. Thus, after a few weeks of synergistic tenotomy, fast muscles become slow, fatigue resistant, and then more adapted to endurance. On the other hand, administration of CO_2 by the rebreathing technique produced an increase in the amplitude of breathing activity, and the respiratory frequency increased due to a decrease in inspiratory and expiratory duration (40). In a human study involving 21 subjects at 15,000 ppm of CO_2 chronic exposure, respiratory acclimatization to CO_2 involved a continuous increase in tidal volume while the respiratory rate declined shortly after an initial increase, associated with an increase in physiological and anatomical dead space (45). Gelhaye et al. showed that orofacial muscles present a profile in MHC adapted to the transition from nasal to oral

breathing, facilitating respiration (17). In the same way, under CO_2, the MHC 2B decreased in the Dia, this muscle adapts its fatigue resistance to the new respiratory frequency.

Conclusion

In conclusion, our work shows that female rats treated with chronic CO_2, an atmospheric pollutant, a situation comparable to the episodes of August 2003 and May 2015 (57) in western European countries, presented modifications of general behaviour, hormonal status (corticosterone), and MHC profile of diaphragm and oral respiratory muscles. Modifications of MHC expression could be induced by various factors, particularly behavioural and hormonal factors. These findings show that the control of atmospheric pollutant levels may be of great importance in the long term considering their incidence on organisms.

In future studies, it would be interesting to analyse the long-term effects of these modifications in adult rats and also other atmospheric pollutants alone and/or in combination, and their effects on studied biological parameters.

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Legends

Table 1. Myosin heavy chain (MHC) distribution in respiratory muscles and in one locomotor muscle in control and CO_2 exposed rats. Values are percentages of total MHC ± SEM (n=12 per group). Statistical summary: ‡ significantly different from control muscles, $p < 0.05$, ND: not detected, NS: not significant.

Figure 1. Behavioural parameters in control and pre-CO_2 exposed rats. Values are means of behavioural events during the week prior to CO_2 exposure (n=12 per group). NS: not significant.

Figure 2. Behavioural parameters in control and CO_2 exposed rats. Values are means of behavioural events during the first day of CO_2 exposure (n=12 per group). Statistical summary (t-student): * significantly different from control, $p < 0.05$, ** significantly different from control, $p < 0.01$, *** significantly different from control, $p < 0.001$. NS: not significant.

Figure 3. Behavioural parameters in control and CO_2 exposed rats. Values are means of behavioural events during the last day of the 15-day period of CO_2 exposure (n=12 per group). Statistical summary: * significantly different from control, $p < 0.05$, ** significantly different from control, $p < 0.01$, *** significantly different from control, $p < 0.001$. NS: not significant.

Figure 4. Plasma levels of corticosterone, free triiodothyronine (FT3) and free thyroxine (FT4) in control and CO_2 exposed rats. Values are mean ± SEM (n=12 per group). Statistical summary: ‡ significantly different from control, $p < 0.05$.

Figure 5. Example of Myosin Heavy Chain 2A, 2X, 2B and 1 isoform migration in diaphragm muscle from control and CO_2 exposed rats.

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	MHC type (%)			
	slow		fast	
Group	**1**	**2A**	**2X**	**2B**
		Diaphragm (Dia)		
control	26.7 ± 0.1	22.6 ± 0.1	31.4 ± 0.2	19.3 ± 0.2
CO_2	25.7 ± 0.1 ‡	24.3 ± 0.4 ‡	31.7 ± 0.1 NS	18.3 ± 0.2 ‡
		Masseter Superficialis (MS)		
control	ND	23.9 ± 0.5	47.8 ± 0.2	28.3 ± 0.5
CO_2	ND	24.4 ± 0.2 NS	46.7 ± 0.1 ‡	28.9 ± 0.3 NS
		Anterior Digastric (AD)		
control	ND	22.7 ± 0.5	32.6 ± 0.2	44.7 ± 0.4
CO_2	ND	23.0 ± 0.2 NS	33.3 ± 0.1 ‡	43.7 ± 0.1 NS
		Levator Nasolabialis (LN)		
control	ND	ND	28.8 ± 0.3	71.2 ± 0.3
CO_2	ND	ND	29.4 ± 0.3 NS	70.6 ± 0.3 NS
		Soleus (S)		
control	84.2 ± 0.3	15.8 ± 0.3	ND	ND
CO_2	87.6 ± 0.2 ‡	12.4 ± 0.2 ‡	ND	ND

Figure 1. Behavioural parameters in control and pre-CO_2 exposed rats. Values are means of behavioural events during the week prior to CO_2 exposure (n=12 per group). NS: not significant.

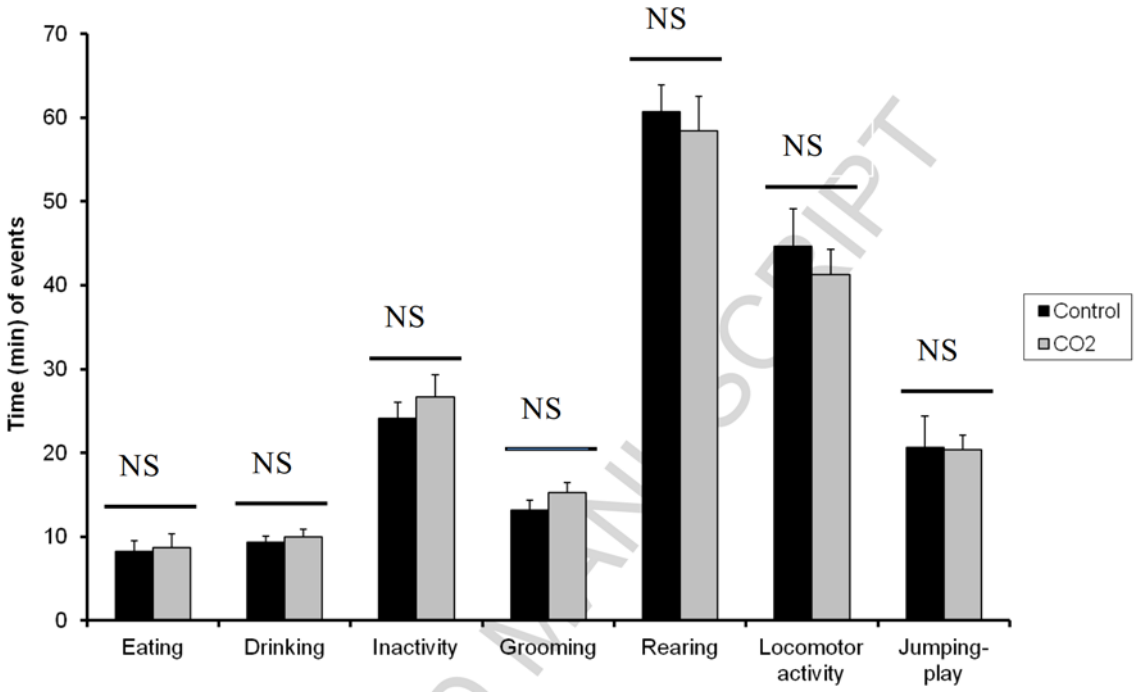
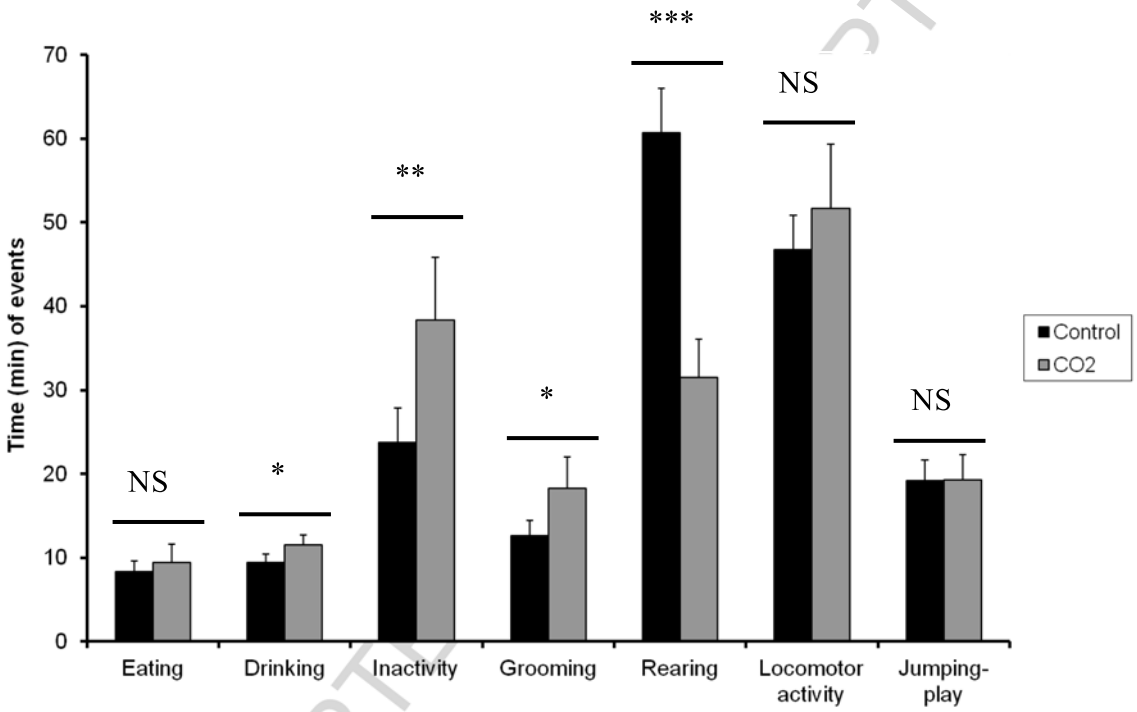


Figure 2. Behavioural parameters in control and CO_2 exposed rats. Values are means of behavioural events during the first day of CO_2 exposure (n=12 per group). Statistical summary (t-student): * significantly different from control, $p < 0.05$, ** significantly different from control, $p < 0.01$, *** significantly different from control, $p < 0.001$. NS: not significant.



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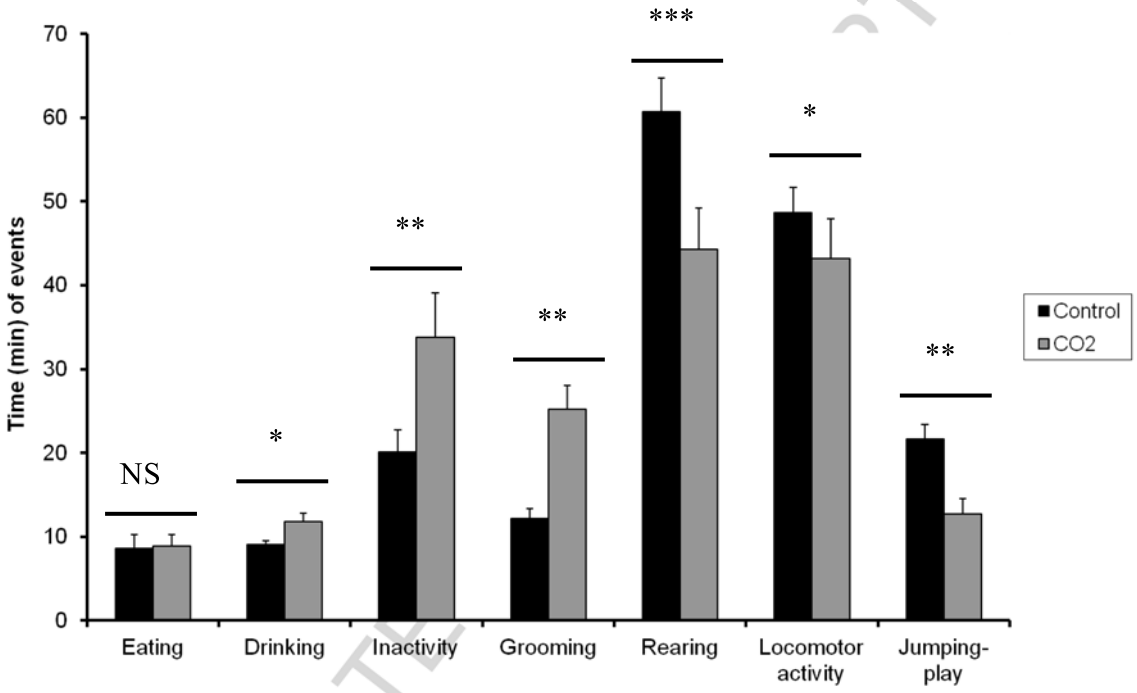

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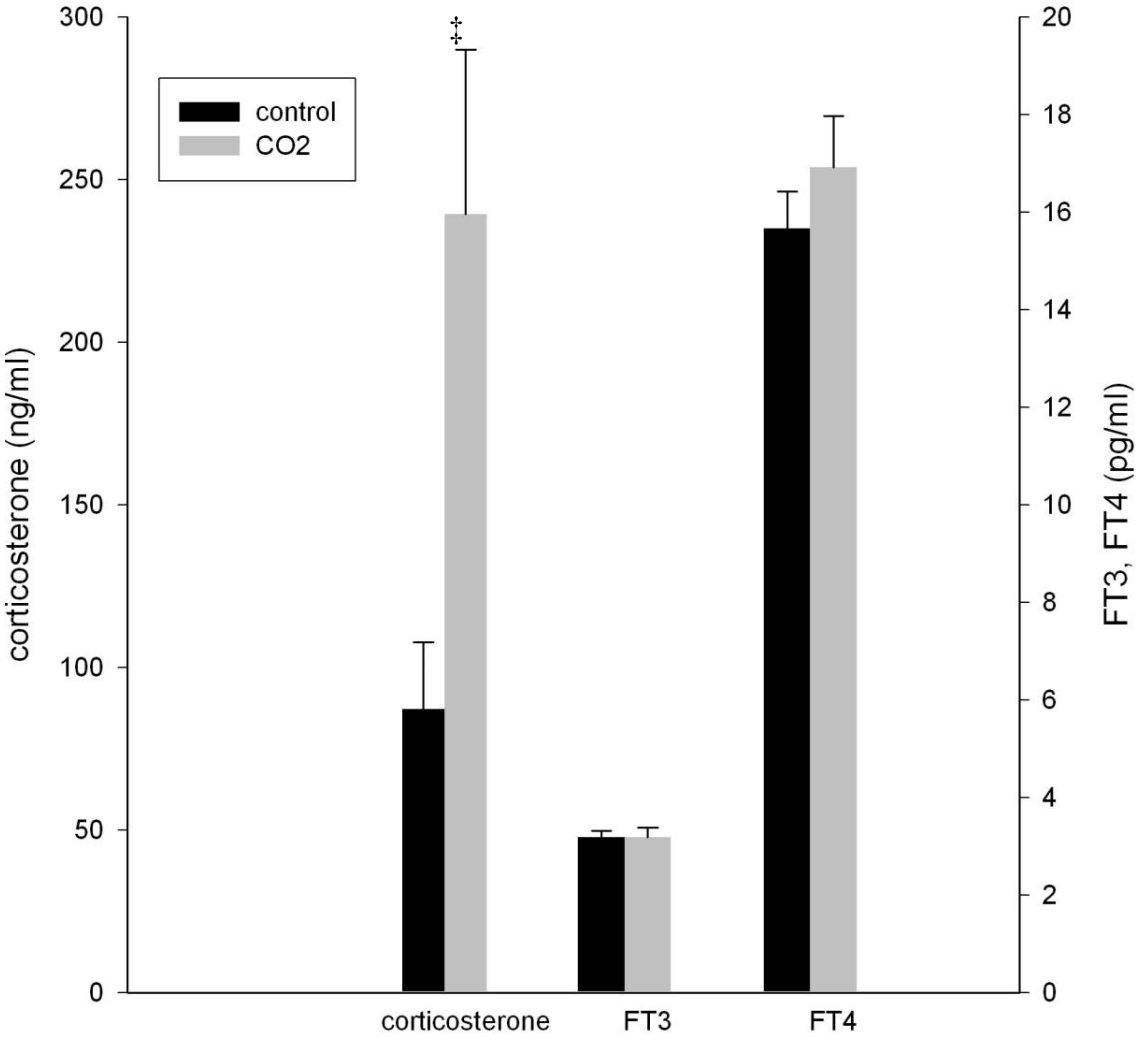

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