

# Acute methamphetamine exposure induces long-lasting depressive-like behaviour in mice

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## Abstract

Methamphetamine (METH) abuse leads to cognitive and mood abnormalities including depressive symptoms. A single high dose of METH administration to rodents models the monoaminergic dysfunction seen in METH addicts. However, whether this neurotoxic METH regimen induces a depressive-like behaviour remains unknown. Herein, we aimed to assess the depressive-like behaviour in mice following a single-high dose of METH. Adult C57BL/6 mice (3-4 month-old) were injected with METH (30 mg/kg, i.p.) and their depressive-like responses were evaluated up to 49 days post-injection in the tail-suspension test (TST). Striatal-related behavioural alterations including motor function and procedural memory were also assessed by pole-test (PT) and Morris water maze (MWM, cued version), respectively. Frontal cortical and striatal changes in monoamine homeostasis as well as glial fibrillary acidic protein (GFAP) levels were evaluated by HPLC-ED and western-blotting. All values were expressed as means  $\pm$  SEM and statistical analysis were performed by Student's t test or by ANOVA followed by Newman-Keuls multiple comparison test. This

single high METH dose caused an acute hyperthermia peaking at 1 h. METH increased significantly the immobility time in the TST at 3, 8 and 49 days post-treatment. In contrast, METH did not disrupt the motor function as evaluated in the PT at 3 and 8 days post-treatment. Additionally, METH did not impair the procedural learning and memory over the four testing days in the cued version of the MWM (1-4 days post-treatment). Moreover, swimming speed was not changed in METH treated mice, reinforcing the absence of motor impairments. Concerning the neurochemistry parameters, a marked depletion of dopamine (30%) and tyrosine hydroxylase (40%) were observed in frontal cortex and striatum of METH-injected mice at 3 and 49 days post-treatment. At 3 days post-treatment, METH also induced a depletion of 5-HT in frontal cortex that persisted up to 49 days. Moreover, a robust astrogliosis observed in the striatum at 3 days lends additional credence to this neurotoxic model.

Our results provide new evidence that an acute administration of METH induces a long-lasting depressive-like behaviour underlined by a long-term monoaminergic disruption in mice. This experimental model of long-term depression-like behaviour is of relevance to unravel the neurobiological substrate underlying depressive symptoms seen in METH addicts.

**Keywords:** Methamphetamine; Tail suspension test; Long-lasting depressive-like behaviour; Monoaminergic disruption; Striatum; Frontal cortex.

## 1. Introduction

Methamphetamine (METH), while being a significant drug problem in North America and East and Southeast Asia since the past decade, has become a more prominent part of the European drug scene, especially in East European countries (Czech Republic, Slovakia and Germany).<sup>1</sup> METH is a highly potent releaser of monoamines, thus increasing extracellular levels of dopamine (DA) and serotonin (5-HT) as well as noradrenaline, through a non-exocytotic mechanism.<sup>2-4</sup>

Chronic METH abuse leads to neurotoxicity and cognitive/motor impairments.<sup>5,6</sup> In fact, post-mortem analysis from chronic METH users have revealed reduced levels of dopaminergic nerve terminal markers [DA, tyrosine hydroxylase (TH), and dopamine transporter (DAT)] in the caudate-putamen as well as in the nucleus accumbens<sup>7-9</sup> and reductions in serotonin transporter (SERT) levels in the orbitofrontal and occipital cortices.<sup>10</sup> These neuropathological changes are, at least in part, associated with the psychiatric features seen in METH chronic users such as anxiety, aggressiveness, social isolation, psychosis, mood disturbances, and psychomotor dysfunction including working memory deficits.<sup>11-14</sup> It was reported that chronic METH abusers showed 5-HT depletion and depressive symptoms.<sup>6,15-19</sup> Interestingly, it was recently showed that rats exhibited a depressive-like state during early withdrawal after compulsive METH intake.<sup>20</sup> However, the prevalence of depression diagnoses in METH addicts has been poorly investigated. Clinical data evidenced that depression may also be related to reduced brain DA levels and its metabolite homovanillic acid (HVA).<sup>10,21-23</sup> Additionally, the disruption of some behaviours (motor speed, motivation, reward, and cognition) observed in depressive pattern has been associated with dopaminergic dysfunction.<sup>24</sup>

Rodent models using acute high-dose METH treatments recapitulate the detrimental effects reported on METH users including hyperthermia, striatal dopaminergic and glial dysfunction.<sup>25–34</sup>

Herein we propose to investigate for the first time the depression-related phenotype in mice associated with a single high-METH injection. Our results clearly show that a high dose of METH (30 mg/kg, i.p.) induces a long-lasting depressive-like behaviour in mice evaluated in the tail suspension test underlined by a long-term dopaminergic/serotonergic disruption. This experimental model is instrumental to pinpoint the neurobiological substrate underlying depressive symptoms seen in METH addicts.

## **2. Materials and methods**

### **2.1 Animals**

Male adult C57BL/6J mice (3–4 months old; 20–28 g; Charles River Laboratories, Barcelona, Spain) were housed 4 per cage, under controlled environmental conditions (12-h light/dark schedule, at room temperature of 23±1 °C, with food and water supplied *ad libitum*). All experiments were approved by the Institutional Animal Care and Use Committee from Faculty of Medicine, Coimbra University, and were performed in accordance with the European Community directive (2010/63/EU). All efforts were made to minimize animal suffering and to reduce the number of animals used. The ARRIVE guidelines have been followed.

## **2.2 Drugs and chemicals**

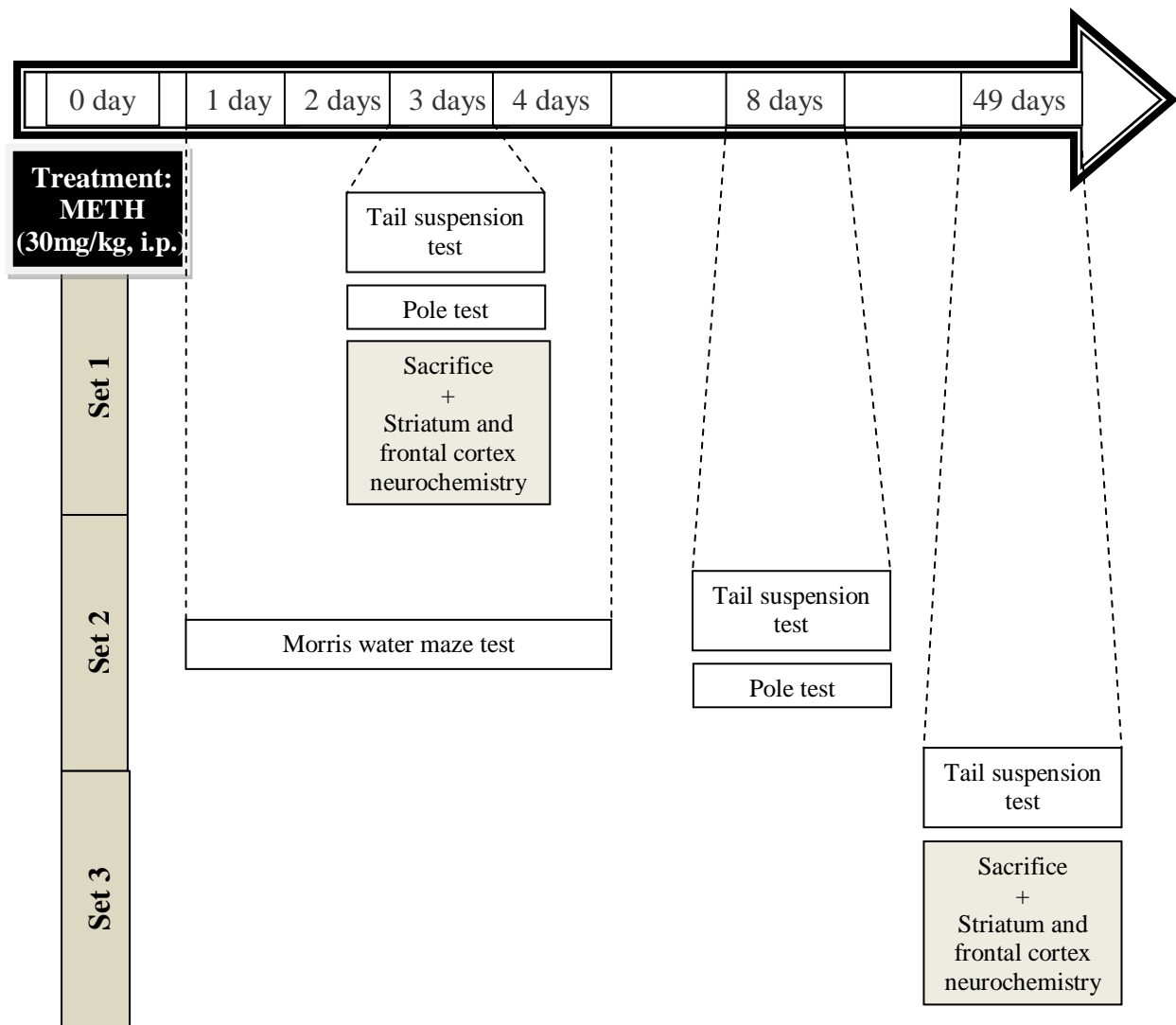
We were issued permission to import METH. HCl from Sigma-Aldrich (St. Louis, MO, USA) by INFARMED, Portugal (National Authority of Medicines and Health Products). Standards for DA, DOPAC, HVA, 5-HT were purchased from Sigma-Aldrich. The other used chemicals (*ultrapure* and *pro analysis* quality) were purchased from Sigma-Aldrich and Merck AG (Darmstadt, Germany).

## **2.3 Drug administration**

Animals were injected intraperitoneally with a single-dose of METH (30 mg/kg) or with saline solution (0.9% NaCl). This is a neurotoxic METH regimen previously established by our research group.<sup>32</sup> Body temperature was assessed with a rectal probe (BAT-12, Physitemp Instruments Inc., Clifton NJ, USA) every 30 min following injection, up to 4 h and at 24 h post-injection.

## **2.4 Behavioural tests**

As illustrated in Figure 1, during a period of 1–49 days after METH (30 mg/kg, i.p.) or control, the behavioural tests were conducted in three independent cohorts of animals and included water maze, tail suspension and pole tasks. Tests were performed in the listed order, from the least aversive to the most aversive, to minimize the chance that behavioural responses would be markedly altered by prior test history.<sup>35</sup>



**Figure 1-** Schematic flow chart of experimental design.

All tests were carried out between 9:00 and 17:00 h and they were scored by the same rater in an observation room where the mice had been habituated for at least 1 h before the beginning of the tests. Behaviour was monitored through a video camera positioned above the apparatuses and the images were later analyzed with the ANY Maze video tracking (Stoelting Co., Wood Dale, IL, USA) by an experienced experimenter who was unaware of the experimental group of the animals tested.

The first set of animals ( $n= 7$  and  $8$ ; controls and METH respectively) performed tail suspension and pole tests at 3 days post-METH.

The second set of animals ( $n= 8$  per group) performed Morris water maze on days 1-4 following METH injection. This group also performed tail suspension and pole tests at 8 days post-METH treatment.

The third set of animals ( $n= 8$  per group) performed the tail suspension test at 49 days following METH injection

#### **2.4.1 Water maze task: procedural memory version**

The used apparatus was made of black painted fibreglass ( $97 \times 60 \times 60$  cm<sup>3</sup>), and the water was maintained at  $23 \pm 2^\circ\text{C}$ . Four distant cues ( $55 \times 55$  cm<sup>2</sup>) were placed 30 cm above the upper edge of the water tank. The target platform ( $10 \times 10$  cm<sup>2</sup>) of transparent acrylic resin was submerged 1–1.5 cm beneath the water surface. Starting points were marked on the outside of the pool as north (N), south (S), east (E), and west (W). They were positioned with the lower edge 30 cm above the upper edge of the water tank, the position of each symbol marked the midpoint of the perimeter of a quadrant (circle = NE quadrant, square = SE quadrant, cross = SW quadrant, and diamond = NW quadrant). A monitor and a video-recording system were installed in an adjacent room.

The animals were submitted to a cued version of the water maze as previously described by Prediger et al. (2006).<sup>36</sup> This consisted of 4 training days, four consecutive trials per day, during which the animals were left in the tank facing the wall, then being allowed to swim freely to the submerged platform placed in the centre of one of the four imaginary quadrants of the tank. The submerged platform was cued by a 7 cm diameter white ball attached to the top of the platform and protruding above the water. The initial position in which the animal was left in the tank was one of the four vertices of the imaginary quadrants of the tank, and this was varied among trials in a pseudo-random way. If a mouse did not find the platform during a period of 60 s, it was gently guided to it. After the animal had escaped

to the platform, it was allowed to remain on it for 10 s and was then removed from the tank for 20 s before being placed in the next random initial position. The swimming speed was calculated averaging the speed values obtained in each trial/day using image analyser (CEFET, Cutitiba, PR, Brasil).

#### **2.4.2 Tail suspension test**

The tail suspension test has become one of the most widely used tests for assessing antidepressant-like activity in mice. It is based on the fact that animals subjected to the short-term inescapable stress of being suspended by their tail, will develop an immobile posture. The total duration of immobility induced by tail suspension test was measured according to the method described by Steru *et al.* (1985).<sup>37</sup> Briefly, mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period. Mice were considered immobile only when they hung passively and completely motionless. The immobility time was recorded by an observer blind to the drug treatment.

#### **2.4.3 Pole test**

The pole test was performed as previously described<sup>38</sup> with minor modifications.<sup>39</sup> It consisted of a 55 cm high wooden pole, 0.8 cm in diameter, wrapped in gauze to prevent slipping and the base position in the home cage. The mouse was placed head-upward on the top of a vertical rough-surfaced pole and the time until it descended to the floor (descent time) was recorded with a maximum duration of 60 s. Even if the mouse descended part way and fell the rest of the way, the behavior was scored until it reached to the floor. When the mouse was not able to turn downward and instead dropped from the pole, descent time was taken as 60 s (default value) because of the maximal severity.



## **2.5 Neurochemistry**

Animals were sacrificed by decapitation at 3 and 49 days post-treatment and striata and frontal cortices were dissected on ice and stored at -80 °C until further analyses. Left brain areas were used for the determination of monoamine (DA, DOPAC, HVA and 5-HT) contents by HPLC-ED, and right areas were used for the quantification of protein expression by Western-blot. In the present study, all animals survived this dosing regimen and none showed convulsions or weight reductions.

### **2.5.1 Monoamine assessment by HPLC-ED**

Left striata and frontal cortices were sonicated in ice-cold 0.2 M perchloric acid and centrifuged (15.500×g, 7 min, 4°C). Supernatants were filtered (9000×g, 10 min, 4 °C) using 0.2 µm Nylon microfilters (Spin-X<sup>®</sup> Centrifuge Tube Filter) and stored at -25 °C until further analyses. The pellet was resuspended in 1 M NaOH and stored at -80 °C for total protein quantification by the bicinchoninic acid (BCA) protein assay (Thermo Fisher Scientific, MA, USA). A Gilson HPLC system was used to determine DA, DOPAC, HVA and 5-HT concentrations in striatum as well as in frontal cortex as described previously.<sup>4</sup> These compounds were separated on a reversed-phase Waters Spherisorb ODS2 column (24.6 mm×250 mm; 5 µm) with a mobile phase (pH=3.8) consisting of 0.1 M sodium acetate trihydrate, 0.1 M citric acid monohydrate, 0.5 mM sodium octane sulphonate, 0.15 mM EDTA, 1 mM dibutylamine and 10% methanol (vol/vol). A flow rate of 1.0 mL/min was maintained for 60 min, and detection of the chromatographed compounds was achieved using a glassy carbon working electrode set at 0.75 V. Sensitivity was set at 2 nA/V. Monoamine concentration was determined by comparison with peak areas of standards, and expressed in nanogram per mg of protein.

## 2.5.2 Western blotting analysis

For measuring TH and GFAP levels, total extracts were obtained as previously described by Simões *et al.* (2008).<sup>40</sup> Right striata and frontal cortices were homogenized in lysis buffer (50 mM Tris-HCl pH 7.4/0.5% Triton X-100, 4 °C), supplemented with a protease inhibitor cocktail (1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, 1 µg/mL chymostatin, 1 µg/mL leupeptin, 1 µg/mL antipain and 5 µg/mL pepstatin A; Sigma-Aldrich) and centrifuged (15,500×g, 15 min, 4 °C) to discard insoluble material. Total protein concentration was determined using the BCA method,<sup>41</sup> and supernatants were stored at -80 °C until further use. Equal amounts of protein (10 µg - TH, GFAP) were loaded and separated by electrophoresis on sodium dodecyl sulphate polyacrylamide gel electrophoresis (12%), transferred to a polyvinylidene difluoride membrane (Millipore, Madrid, Spain), and blocked with 5% non fat dry milk in phosphate buffer saline for 1 h at room temperature. The membranes were probed with mouse anti-TH (1:5000; Millipore, MA, USA) and mouse anti-GFAP (1:5000; Millipore) overnight at 4 °C. Membranes were then incubated with alkaline phosphatase-conjugated secondary antibodies (1:10,000 anti-mouse, anti-goat – Sigma-Aldrich – or anti-rabbit — GE Healthcare, USA). Finally, membranes were visualized on a Storm 860 Gel and Blot Imaging System (GE Healthcare, Buckinghamshire, UK), using an enhanced chemifluorescence detection reagent (ECF, GE Healthcare). To confirm equal protein loading and sample transfer, membranes were reprobed with β-tubulin (1:10,000; Sigma-Aldrich) or GAPDH (1:5000; Abcam, Cambridge, UK) antibodies. Densitometric analyses were performed using the Image Quant 5.0 software. Results were normalized against β-tubulin or GAPDH, and then expressed as percentage of control.

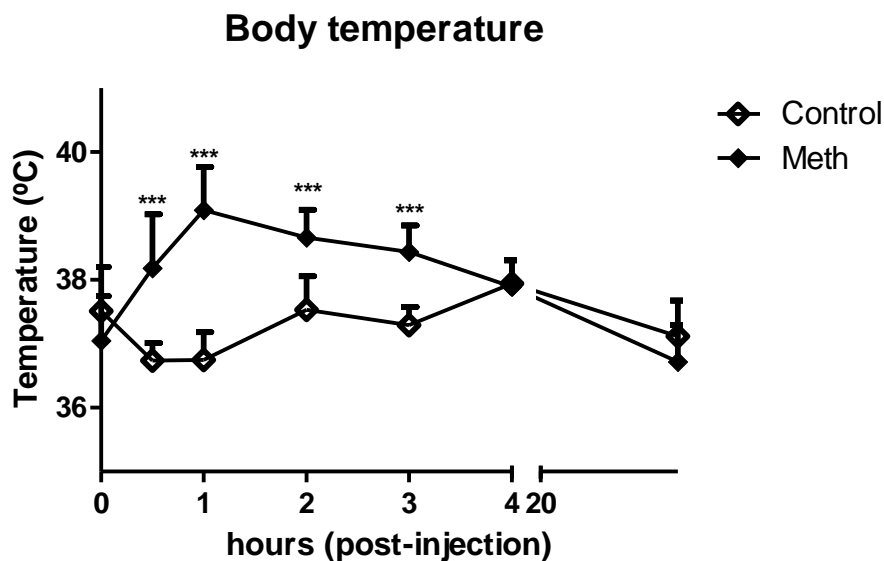
## 2.6 Statistical analysis

The data are expressed as means  $\pm$  S.E.M.. Body temperature and Morris water maze data were analyzed by one-way ANOVA followed by the Newman-Keuls multiple comparison test. Data from the other experiments were analyzed by using unpaired Student's t-test. Significant differences were defined at  $P < 0.05$ . All analyses were performed using GraphPad Prism 5.0 software for Windows.

## 3. Results

### 3.1 Effects of METH on body temperature in mice

Animals treated with a single high dose of METH (30 mg/kg, i.p.) displayed hyperthermia peaking at 1 h and returning to normal levels at 4 h. Twenty-four hours following METH injection the temperature remained normal (Fig. 2). This transient hyperthermia is consistent with previous findings described by our group<sup>32</sup> and others<sup>42</sup> using the same METH dose.



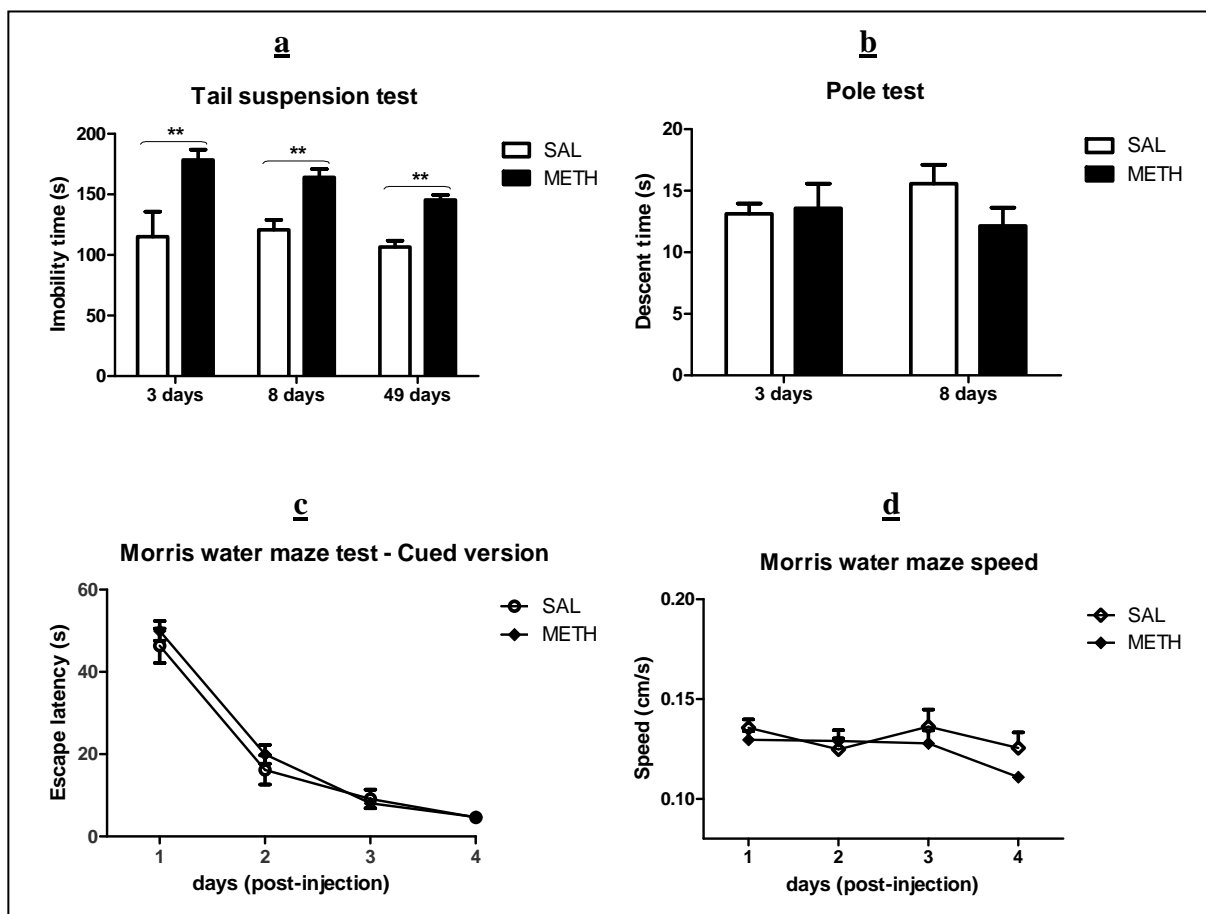
**Fig. 2** - Effects of a single METH administration (30 mg/kg, i.p.) on body temperature (intra-rectal) of mice during a period of 24 hours post-injection. The results are expressed as mean  $\pm$  S.E.M. of 16 animals per group (Sets 1 and 2). \*\*\* $P < 0.001$  Statistics were performed by using one-way ANOVA followed by the Newman-Keuls test.

### **3.2 METH induces long-lasting depressive-like behaviour in mice**

The mice treated with a single high dose of METH (30 mg/kg, i.p.) were evaluated on the tail suspension test at 3, 8 and 49 days post-injection. The METH group showed an increased immobility time on tail suspension test at the three evaluated time-points when compared with SAL group ( $P < 0.01$ ) (Fig. 3a).

### **3.3 Effects of METH on striatal-dependent behavior in mice**

As illustrated in Fig. 3b, METH-injected mice exhibited normal motor coordination when compared with SAL group as evaluated in the pole test at 3 and 8 days. Moreover, no significant differences between METH and SAL groups in latency time to reach the cued platform were observed in the Morris water maze task at 1, 2, 3 and 4 days post METH-injection (Fig. 3c). Additionally, the swimming speed during the performance of the Morris water maze was recorded and no significant differences between groups were detected (Fig. 3d).

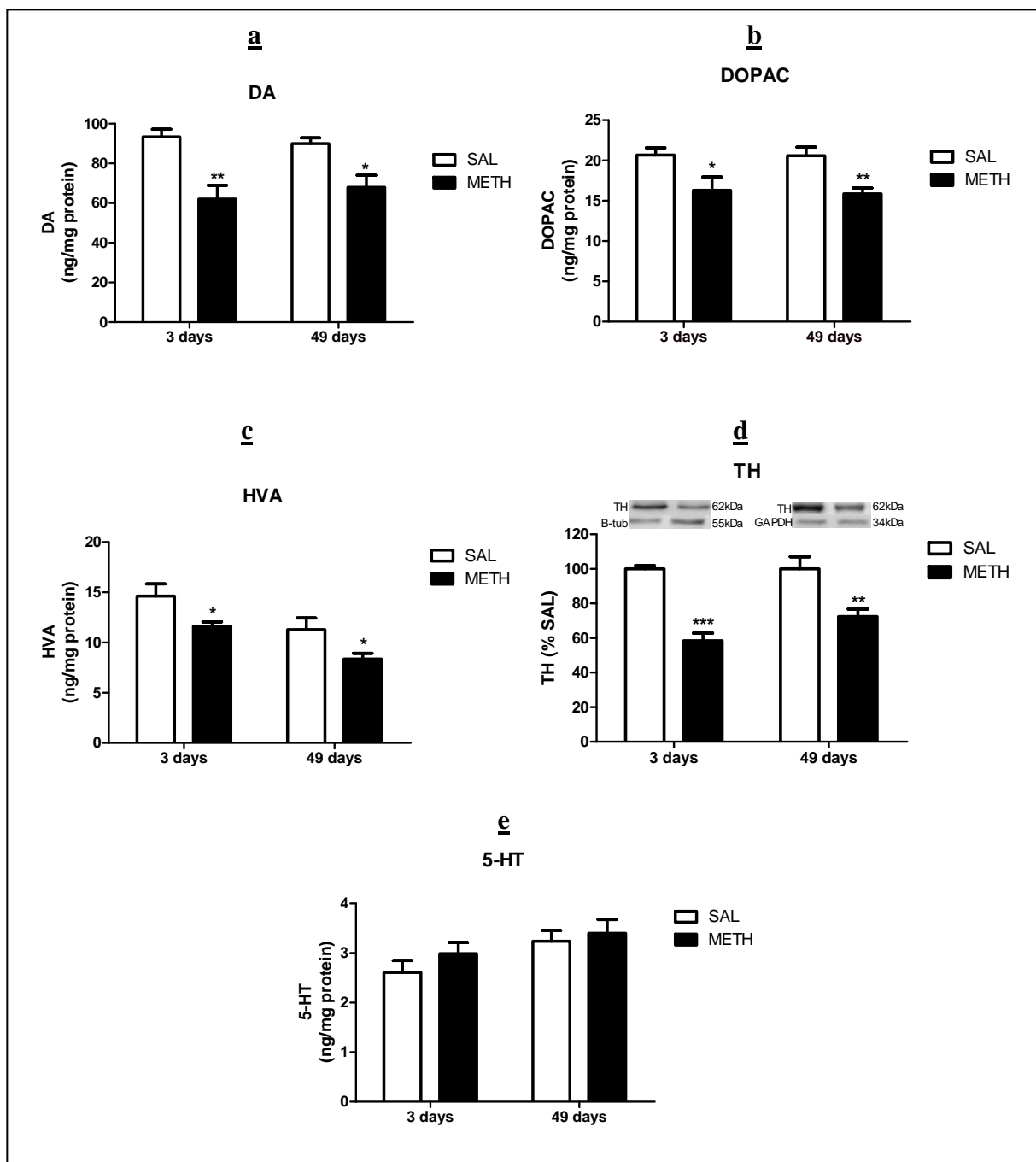


**Fig. 3** - The effects of a single dose of METH (30 mg/kg, i.p.) on the behavioural performance of mice evaluated in three behavioural tests dependent of striatal function: tail suspension, Morris water maze (cued version) and pole tests. The values represent: the immobility time on the tail suspension test 3 8 and 49 days post-injection (a); the descent time on the pole test 3 and 8 days post-injection (b); the latency for escape to a cued platform and swimming speed on the Morris water maze test – cued version 1, 2, 3 and 4 days post-injection (c and d). Escape latencies (s) and swimming speed (cm/s) for the individual trials were averaged by day. The results are expressed as mean  $\pm$  S.E.M. of 7 - 8 animals per group. **\*\*** $P < 0.01$  versus saline (SAL)-treated animals. Statistics were performed by using unpaired Student's t-test (a and b) and one-way ANOVA with Newman-Keul's post-test (c and d).

### 3.4 Effects of METH on striatal monoamine homeostasis

The effect of a single injection of METH (30 mg/kg, i.p.) on DA and its metabolites content in striatum is seen in Fig. 4(a, b, c, d). As expected, METH produced a significant depletion of DA and its metabolites, DOPAC and HVA, in the striatum at 3 days (DA, 33%;

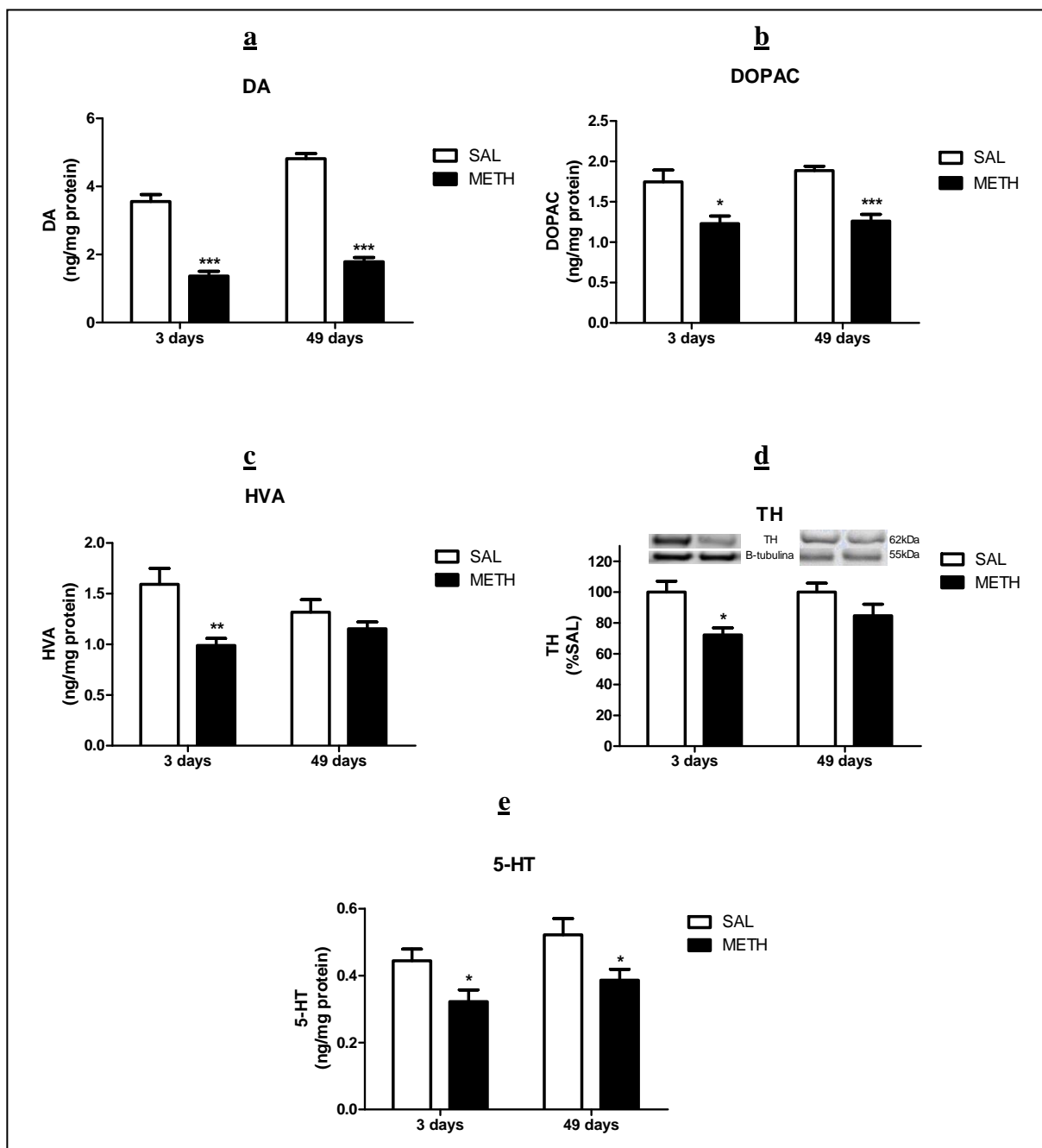
DOPAC, 20%; HVA, 20%;  $P < 0.05$ ) that persisted at 49 days post-injection (DA, 25%; DOPAC, 23%; HVA, 27%;  $P < 0.05$ ). METH also produced a marked reduction in TH levels (41%;  $P < 0.001$ ) at 3 days that continues at 49 days after treatment (28%;  $P < 0.01$ ), as shown in Fig. 4d. We also analyzed the impact of METH on striatal levels of 5-HT. Nevertheless, METH did not significantly change 5-HT levels in striatum neither at 3 nor at 49 days (Fig. 4e) ( $P > 0.05$ ). This is consistent with our previous results.<sup>32</sup>



**Fig. 4** - The effects of a single dose of METH (30 mg/kg, i.p.) on striatal monoamine homeostasis. (a) DA, (b) DOPAC and (c) HVA striatal tissue contents (HPLC-ED) were significantly decreased 3 and 49 days following METH. (d) TH expression (Western Blot) was also significantly decreased at these end-points. (e) 5-HT levels (HPLC-ED) did not differ at both time-points. \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ , versus SAL-treated animals. The results are expressed as mean percentage of SAL  $\pm$  S.E.M. of 6 animals per group. Statistics were performed by using unpaired Student's t-test.

### 3.5 METH impact in frontal cortical monoamine homeostasis

The effect of a single injection of METH (30 mg/kg, i.p.) on DA and its metabolite content in frontal cortex is seen in Fig. 5 (a, b, c, d). METH produced an important depletion of DA and its metabolites, DOPAC and HVA, in the frontal cortex at 3 days (DA, 61%; DOPAC, 29%; HVA, 37%;  $P < 0.05$ ) that persist at 49 days post-injection (DA, 62%; DOPAC, 33%;  $P < 0.05$ ) (Fig. 5a, b, c). The reduction in HVA levels seen at 49 days post-METH did not attain statistical significance. Concordantly, METH also produced a significant reduction in TH levels (28%;  $P < 0.05$ ) at 3 days. Although TH levels were still reduced at 49 days after treatment, this reduction did not reach statistical significance, as shown in Fig. 5d. The impact of METH on frontal cortical levels of 5-HT was evident by the reduction observed both at 3 (27%) and 49 days after treatment (25%) (Fig. 5b) ( $P < 0.05$ ).

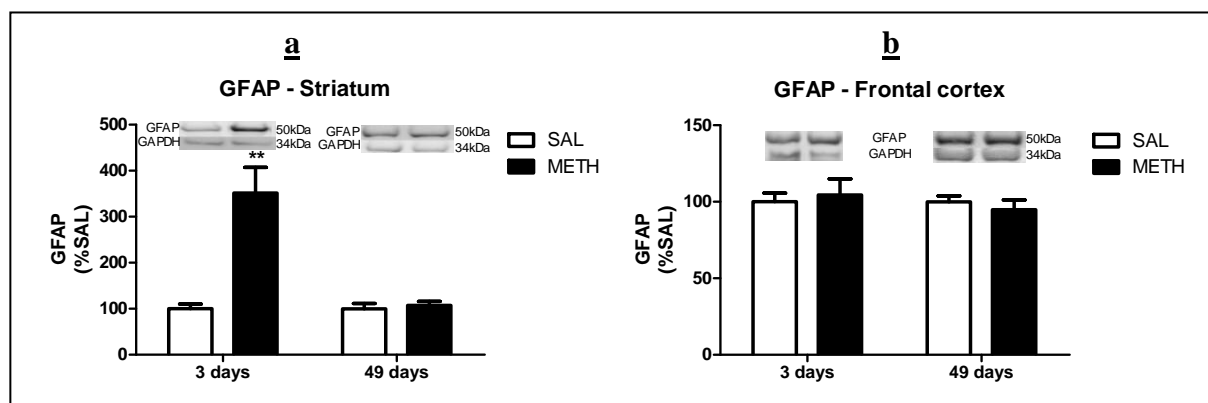


**Fig. 5-** The effects of a single dose of METH (30mg/kg, i.p.) on monoamine levels from frontal cortex. (a) DA, (b) DOPAC and (c) HVA cortical tissue contents (HPLC-ED) were significantly decreased 3 and 49 days following METH. (d) TH expression (Western Blot) was also significantly decreased at 3 days post-treatment but not at 49 days. (e) 5-HT levels (HPLC-ED) were significantly decreased 3 and 49 days post METH-treatment. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , versus SAL-treated animals. The results are expressed as mean percentage of SAL  $\pm$  S.E.M. of 6 animals per group. Statistics were performed by using unpaired Student's t-test.



### 3.6 Effects of METH on GFAP levels in frontal cortex and striatum

Striatal astrogliosis (examined by GFAP labeling) was significantly increased 72 h post-METH, as compared to SAL controls (350% of SAL;  $P < 0.01$ ) (Fig. 6a). However, striatal GFAP levels were normal at 49 days post-METH. On the other hand, METH failed to induce astrogliosis in frontal cortex at any studied time-point (Fig. 6b). This lack of cortical effect may reflect technical limitations. In fact O'Callaghan and Miller (1994) detected a small cortical GFAP increase 3 days post-acute METH treatment using ELISA.<sup>27</sup>



**Fig. 6** - The effects of a single dose of METH (30 mg/Kg, i.p.) on GFAP levels (Western-blot) on Striatum (a) and Frontal cortex (b) of mice. METH increased GFAP protein levels 3 days post-injection in the striatum (a) \*\*  $P < 0.01$ . Data are presented as means  $\pm$  S.E.M. ( $n = 6$  animals per experimental group). Statistics were performed by using unpaired Student's t-test.

## 4. Discussion

Our results show, for the first time, that a single injection of a high dose of METH (30 mg/kg, i.p.) triggered a pronounced long-lasting depressive-like behaviour in mice, concurrent with long-term dopaminergic and serotonergic disruption on frontal cortex and striatum. This METH regimen consistently induced neurotoxicity as seen by hyperthermia and striatal astrogliosis herein.<sup>32</sup>

Clinical studies showed psychomotor slowing and depressive symptoms in abstinent METH abusers.<sup>19,43,44</sup> Giving emphasis on the depressive phenotype, it is worthwhile mentioning by Brière *et al.*, (2012)<sup>16</sup> that adolescent consumption of amphetamines including METH was associated with subsequent depressive symptoms.

However, the behavioural profile of laboratory rodents after an acute single neurotoxic METH dose has been overlooked. Nevertheless, Pereira *et al.*, (2012)<sup>32</sup> showed that 30 mg/kg METH decreased spontaneous locomotor activity of mice in the open-field test during 1-h sessions, at 24 and 48 hours after METH administration. Interestingly, Paulson *et al.*, (1991)<sup>45</sup> as well as Kokkinidis *et al.*, (1986)<sup>46</sup> observed that rats experiencing amphetamine-withdrawal exhibited a reduced exploratory behaviour on the open-field test without locomotor impairment. These authors suggested that this hypolocomotion reflected a low motivation and affective state that may be related to a depression-like behaviour.

We clearly showed that METH (30 mg/kg, i.p.) increased the immobility scores at 3, 8 and 49 days post-injection using tail suspension test, which is indicative of a long-lasting depressive-like behaviour. Interestingly, it was also shown that a sub-chronic neurotoxic regimen of another amphetamine-derivative compound [3,4-methylenedioxymethamphetamine, MDMA] induced a long-term depressive-like behaviour in mice evaluated in the forced swimming test.<sup>47</sup>

On the other hand, Jang et al. (2013)<sup>20</sup> described a depressive-like state during early withdrawal following METH self-administration with extended access in rats by using forced swim test. Additionally, rodents administered amphetamine (5-10 mg/kg/day for 7 days using an osmotic pump) exhibited a significant increase in immobility scores in tail suspension test 24 hours following withdrawal.<sup>48</sup>

The lack of alterations in both swimming speed (Morris water maze test – cued version) from 1 to 4 days and pole test at 3 and 8 days demonstrated that the METH-exposed animals have normal motor function, thus ruling out its contribution to the depressive-like behaviour observed within this timeframe. Although motor function was not assessed at 49 days, it is likely that it remained normal at this time-point.<sup>49</sup>

Furthermore, we show that depressive-like behaviour coexists with a long-lasting disruption of monoamines homeostasis in both striatum and frontal cortex. As expected, this METH paradigm imposed striatal and cortical dopaminergic dysfunction, as seen by the degree of DA/metabolites as well as TH depletion, at 3 days. We further demonstrated that this DA depletion remained at 49 days. This long-term striatal dopaminergic disruption is consistent with rodent prolonged striatal dopaminergic dysfunction triggered by an acute METH paradigm in rodent reported by Hotchkiss *et al.* (1979), O’Callaghan and Miller, (1994) and Friedman *et al.* (1998).<sup>27,50,51</sup> However, this striatal dopaminergic disruption was not sufficient to impair the habit learning, since METH-treated mice performed normally in the procedural memory version of the water maze.

We further observed a long-term DA as well as 5-HT cortical depletion in METH-treated mice. Although this is the first time that long-lasting METH-induced cortical DA depletion is shown, 5-HT depletion is in line with Friedman *et al.* (1998)<sup>51</sup> that showed a persistent cortical 5-HT depletion in rat following an acute METH regimen. This lends further support to the neurochemical disturbance underlying a depressive disorder.

In fact, the “monoamines hypothesis” which suggests a deficiency or imbalances in the monoamines as the cause of depression has been the central topic of depression research for approximately the last 50 years.<sup>52</sup> In spite of noradrenaline and serotonin being traditionally envisioned as key players in the aetiology of depressive disorders, there is a growing amount of data focusing on the implication of the dopaminergic system.<sup>53–56</sup> For example, a recent review by Savits and Drevets (2012) suggested that the anhedonia and motivational deficits characteristic of depression may result from decreased dopaminergic signalling in the ventral striatum concurrent with the reduction of dopaminergic markers including TH.<sup>57</sup>

Additionally, very recent data on the critical role of glia-modulating neuronal dysfunction as well as neuroinflammation in depression has being discussed.<sup>58–60</sup> Therefore METH-induced astroglial changes seen herein might also contribute to depressive-like behaviour.

## **5. Conclusion**

Overall, these results, while stressing the neurotoxicity profile of an acute high-dose of METH on monoaminergic system, put in evidence its behavioural consequences, namely the long-lasting “depressive-like behaviour” without pronounced motor impact. This study provides a contribution not only to study human METH abstinence effects but also to elucidate depression disease neurochemistry.

## **Acknowledgments**

We would like to acknowledge Ms. Inês Pita (Laboratory and Pharmacology and Experimental Therapeutics/Biomedical Institute for Research on Light and Image (IBILI), Faculty of Medicine, University of Coimbra) for helping with the behavioural tasks.

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