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**THE EFFECTS OF FOOTSHOCK AND IMMOBILIZATION STRESS ON
TYROSINE HYDROXYLASE PHOSPHORYLATION IN THE RAT LOCUS
COERULEUS AND ADRENAL GLAND**

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Abstract

Tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis, is regulated acutely by protein phosphorylation. No studies have systematically investigated the time course of TH phosphorylation *in vivo* in response to different stressors. We therefore determined the extent of TH phosphorylation at Ser19, Ser31 and Ser40 over a 40 min period in response to footshock or immobilization stress in the rat locus coeruleus and adrenal medulla. There were significant changes in TH phosphorylation in both tissues and the responses to the two stressors differed markedly. With each of the phosphorylation sites immobilization stress caused a much smaller, or less sustained, response than footshock stress. With immobilization stress there was a transient increase in Ser31 phosphorylation in the locus coeruleus at 5 min and in the adrenal medulla at 10 min, but there were no effects on Ser19 or Ser40 phosphorylation. With footshock stress there was a substantial decrease in Ser19 phosphorylation over time, a substantial increase in Ser31 phosphorylation over time, but there were no effects on Ser40 phosphorylation. The effects of these stressors on the phosphorylation of TH at each of the sites are interpreted in respect to the relative activation of the locus coeruleus and the adrenal medulla cells and the activation of TH.

Key words: Tyrosine hydroxylase, Serine residues, Locus Coeruleus, Adrenal Medulla, Footshock, Immobilization

Abbreviations: LC, Locus coeruleus; AM, Adrenal medulla; TH, Tyrosine hydroxylase; Ser, Serine residue; FS, Footshock; IMO, Immobilization

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Stress induces activation of central catecholaminergic neurons, the peripheral sympathetic nervous system and the adrenomedullary system (Kvetnansky et al., 2009). Exposure to stress leads to an increase in the release of catecholamines from these neurons and chromaffin cells. One of the major central catecholaminergic systems that is involved in the stress response is the ascending noradrenergic system, originating primarily in the A6 noradrenergic neurons of the locus coeruleus (LC) (Morilak et al., 2005). Stress stimulates electrical activity in the LC and the release of noradrenaline onto forebrain target sites (Smith et al., 1991; Chang et al., 2000). The firing rate of LC neurons has been shown to be increased by footshock and immobilization stress (Stanford, 1995). Likewise, chromaffin cells of the adrenal medulla (AM) produce noradrenaline and adrenaline which are released into the circulatory system in response to splanchnic nerve activation (Wakade, 1981). The firing rate of splanchnic nerve has been shown to be increased by various stressors (de Diego et al., 2008; Kvetnansky et al., 2009).

When catecholamines are released from the LC or the AM there is a compensatory increase in catecholamine synthesis. This maintains the cellular levels of catecholamines in these tissues constant (Wakade et al., 1988). The rate limiting enzyme in catecholamine synthesis is tyrosine hydroxylase (TH) (Nagatsu et al., 1964; Dunkley et al., 2004). Stress increases TH activity (Kvetnansky et al., 1970) and many different stressors, including footshock (Melia and Duman, 1991; Chang et al., 2000) and immobilization (Nankova et al., 1994), have been shown to induce TH activation in the LC and the AM. TH activation leads to increased catecholamine synthesis to replace released catecholamines. This occurs by two mechanisms. Firstly, the extracellular calcium influx into the cells that leads to the release of the catecholamines, also leads to activation of signal transduction pathways that acutely increase TH phosphorylation and activity (Kumer and Vrana, 1996). Secondly, the activation of signal

transduction pathways also leads to phosphorylation of transcription factors, which over time increases TH mRNA and TH protein levels (Kumer and Vrana, 1996). TH phosphorylation, TH mRNA and TH protein levels are therefore all indicators of catecholamine cell activation in response to stress.

TH phosphorylation at Ser19, Ser 31 and Ser 40 is involved in the acute activation of TH. TH phosphorylation follows a pattern over the first 10 min *in vitro*: Ser19 phosphorylation is rapidly increased to maximal levels within a minute and then dephosphorylation occurs, Ser40 phosphorylation begins more slowly than Ser19 reaching a plateau by 4 min, without any subsequent dephosphorylation, Ser31 phosphorylation is delayed until 4 min but rapidly increases up to 10 min (Haycock, 1993). TH phosphorylation at Ser19 does not increase TH activity directly *in vitro* (Sutherland et al., 1993). TH phosphorylation at Ser31 increases TH activity about 2-fold *in vitro* (Haycock, 2002). TH phosphorylation at Ser40, which relieves the feedback inhibition by catecholamines (Kumer and Vrana, 1996), can increase TH activity up to 40-fold *in vitro*. TH phosphorylation has been shown to correlate with TH activity in cell cultures (Haycock, 1993; Dunkley et al., 2004; Bobrovskaya et al., 2007a; Bobrovskaya et al., 2007b). The profile of TH phosphorylation at Ser19, Ser31 and Ser40 can therefore be used as an indicator of TH activation in response to acute stress. There are studies *in vivo* on changes in TH phosphorylation at Ser19, Ser31 and Ser40 (Jedynak et al., 2002; Dunkley et al., 2004; Bobrovskaya et al., 2010; Ong et al., 2011). However, no studies have systematically investigated the time course of phosphorylation of these sites *in vivo* in response to different stressors.

Many studies have investigated TH mRNA and/or TH protein changes in response to stressors (Watanabe et al., 1995; Rusnak et al., 2001; Tank et al., 2008), but these measures are only

detectable many hours after exposure to the stressor. In the LC, TH mRNA levels were significantly increased 6 hr after a 15 min period of footshock stress (Chang et al., 2000), while TH protein levels were significantly increased 24 hr after a 2 hr period of immobilization stress (Hebert et al., 2005). In the AM, TH mRNA levels were significantly increased only after 2 hr of immobilization stress, whereas TH protein levels were significantly increased only 6 hr after a 2 hr period of immobilization stress (Nankova et al., 1994). TH mRNA cannot be used as an index of TH activation as the level does not necessarily correlate with the level of TH protein. Furthermore, the level of TH protein does not necessarily correlate with the level of TH activation as the enzyme may be synthesized, but be inactive due to catecholamine binding. The levels of TH mRNA or TH protein cannot therefore be used as a direct indicator of TH activation in response to acute stress.

We wanted to investigate the effect of two acute stressors on TH phosphorylation as an indicator of both catecholamine cell activation and TH activation *in vivo*. We therefore determined the level of TH phosphorylation at Ser19, Ser31 and Ser40 over a 40 min period in response to footshock and immobilization in the rat LC and AM.

EXPERIMENTAL PROCEDURES

Materials

Sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE) reagents were purchased from Bio-Rad Laboratories (Hercules, CA, USA). Molecular weight PAGE standards, nitrocellulose membrane (Hybond ECL), ECL plus kit, anti-rabbit immunoglobulin (horseradish peroxidase-linked whole antibody from donkey) and anti-mouse immunoglobulin (horseradish peroxidase-linked whole antibody from sheep) were obtained from GE Health Care (Little Chalfont, UK). EGTA, EDTA, Tween-20, bovine serum albumin, sodium azide, isopentane and β -actin antibody were from Sigma Chemical Co. (St Louis, MO, USA). Anti-sheep antibody (horseradish peroxidase-linked whole antibody from rabbit) was obtained from Pierce Biotechnology (Rockford, IL, USA). Total-TH antibody and phospho-specific TH antibodies (pSer19, pSer31 and pSer40) were generated and tested for specificity according to (Gordon et al., 2009).

Animals

All experiments were approved by the University of Newcastle Animal Care and Ethics Committee and performed in accordance with the New South Wales Animal Research Act and the “Australian code of practice and use of animals for scientific purposes”. Adult male Sprague-Dawley rats (300-400g) were obtained from Animal Resources Centre (ARC), Perth, Australia. Animals were maintained in group housing under standard laboratory conditions in temperature controlled rooms ($21 \pm 1^\circ\text{C}$), reverse 12 h light cycle with darkness from 02:00 to 14:00 h, food and water ad libitum. Animals were habituated and handled for 7 days prior to experiments in order to reduce stress associated with handling.

Animal treatments

Footshock Sham (FS-), animals were placed into a footshock chamber (San Diego Instruments, CA, USA) for 10 min (n=6), 20 min (n=6) or 40 min (n=6). Footshock (FS+), animals were placed into a programmable footshock chamber and received series of footshock (1 mA, 1 sec pulse per minute) for 10 min (n=6), 20 min (n=6) or 40 min (n=6). Immobilization Sham (IMO-), animals were placed into a holding box (30 cm x 20 cm x 20 cm) for 10 min (n=6), 20 min (n=6) or 40 min (n=6). Immobilization (IMO+), animals were physically restrained with wire mesh so that they were unable to turn or move substantially and were placed into a holding box for 10 min (n=6), 20 min (n=6) or 40 min (n=6). Home cage control (HCC), animals remained in their home cages (n=6). Immediately after treatment, animals were administered intraperitoneally with sodium pentobarbital (Lethabarb, 80mg/kg). Animals were decapitated by guillotine once the animals showed a lack of response to painful stimuli (foot pinch) (<5 min). Immediately, the brain and adrenals were dissected and frozen in -80°C in isopentane. Tissues were kept at -80°C until required.

Sample Preparation

Locus coeruleus (LC) was identified by reference to the Paxinos and Watson stereotaxis brain atlas. Sections (Bregma -9.86 to -10.52 mm) were cut at -20°C on a customized freezing microtome (SM2000R, Leica) with an attached digital micrometer and rapidly transferred to a refrigerated working bench held at 4°C. The bilateral LC regions were punched out using a 5 mm bore punch. LC and adrenal tissues were processed as previously described (Ong et al., 2010). Briefly, the LC and adrenal samples were homogenised using a sonicator (Soniprep

150, MSE) in homogenisation buffer (2% SDS, 2 mM EDTA, 50 mM Tris, pH 6.8). Samples were then centrifuged at 15,000 rpm for 20 min at 4°C. The clear supernatants were collected and were mixed with sample buffer (1 % SDS, 10 % glycerol, 0.5 % DTT and minimal bromophenol blue).

SDS-PAGE and Western Blotting

Samples were subjected then to SDS–polyacrylamide gel electrophoresis before being transferred to nitrocellulose (Jarvie and Dunkley, 1995). Membranes were then stained with Ponceau S (0.5% ponceau in 1% acetic acid) to assess the efficacy of the transfer. Membranes then were washed in Tris-buffered saline with Tween (TBST) (150 mM NaCl, 10 mM Tris, 0.075% Tween-20, pH 7.5) and incubated with blocking solution (5% bovine serum albumin, 0.04% sodium azide in TBST) for 2 hr at 25°C. Membranes were washed in TBST and incubated with primary antibodies (total TH, phospho-specific TH and β -actin) for 1 hr at 25°C. Membranes were washed in TBST and incubated with horse-radish peroxidase-linked anti-IgG secondary specific antibodies for 1 h at 25°C. Membranes were visualized on Fujifilm Las-3000 imaging system (Fuji, Stamford, CT, USA) using ECL plus detection reagents. The density of total TH, phospho-specific TH and β -actin bands were measured using a MultiGauge V3.0 (Fuji, Stamford, CT, USA). Total TH protein levels were expressed as the ratio of TH protein to β -actin. β -actin levels were used as house keeping protein. Site-specific TH phosphorylation at Ser19, Ser 31 and Ser40 was expressed as a ratio relative to total TH protein to account for variability in total TH between samples.

Statistical Analysis

The data for FS-, FS+, IMO- and IMO+ groups were expressed as a fold increase of the mean $\pm$ SEM for each group relative to the mean of the HCC group. These data were analysed by using PRISM V4.02 (GraphPad Software, Inc., CA, USA). Two-way ANOVA was used to examine whether there were any significant effects of treatment and/or time across the groups. Additionally, Bonferroni post tests were used to compare between FS- vs FS+ and IMO- vs IMO+ differences at each of the time points. All differences were considered to be significant at $p < 0.05$.

RESULTS

In the AM samples total-TH protein and phospho-TH (pSer19, pSer31 and pSer40) appeared as single bands corresponding to molecular masses of approximately 60 kDA (Figure 1). Similar results were found with the LC (not shown). In this paper the results for TH protein levels were calculated relative to the levels of β -actin (Figure 2) and the results for TH phosphorylation were calculated relative to TH protein levels (Figures 3-5). The results for the FS+, FS-, IMO+ and IMO- groups were presented relative to the HCC group data being set as equal to 1.0.

The effect of footshock or immobilization stress on total-TH protein in the locus coeruleus and the adrenal medulla

Rats were exposed to either footshock (Figures 2A, C) or immobilization stress (Figures 2B, D) for 10, 20 or 40 mins. The LC and AM were collected and TH protein levels were determined. There was no effect of treatment or time on total TH protein expression in the LC or the AM; there was also no significant effect of footshock or immobilization stress at any time point in any of the groups analysed.

The effect of footshock or immobilization stress on TH phosphorylation at Ser19 in the locus coeruleus and the adrenal medulla

Rats were exposed to either footshock (Figures 3A, C) or restraint stress (Figures 3B, D) for 10, 20 or 40 mins. The LC and AM were collected and TH phosphorylation levels at Ser19 were determined. There was a significant effect of treatment ($F=10.28$, $DFn=1$, $p=0.0032$) and

time ($F=15.56$, $DFn=2$, $p<0.0001$) on pSer19 levels in the LC in animals exposed to footshock stress (Figure 3A). Post hoc analysis (Bonferroni post tests), indicated that relative to FS-, FS+ displayed a significant decrease in pSer19 at 40 min ($p<0.05$). There was a significant effect of time ($F=5.24$, $DFn=2$, $p=0.0098$) on pSer19 levels in the LC in animals exposed to immobilization stress (Figure 3B). There was a significant effect of treatment ($F=45.66$, $DFn=1$, $p<0.0001$) and time ($F=129.00$, $DFn=2$, $p<0.0001$) on pSer19 levels in the AM in animals exposed to footshock stress (Figure 3C). Post hoc analysis, indicated that relative to FS-, FS+ displayed a significant decrease in pSer19 at 10 min ($p<0.001$) and 20 min ($p<0.01$). There was a significant effect of time ($F=18.86$, $DFn=2$, $p<0.0001$) on pSer19 levels in the AM in animals exposed to immobilization stress (Figure 3D).

The effect of footshock or immobilization stress on TH phosphorylation at Ser31 in the locus coeruleus and the adrenal medulla

Rats were exposed to either footshock (Figures 4A, C) or restraint stress (Figures 4B, D) for 10, 20 or 40 mins. The LC and AM were collected and TH phosphorylation levels at Ser31 were determined. There was a significant effect of treatment ($F=64.00$, $DFn=1$, $p<0.0001$) and time ($F=4.56$, $DFn=2$, $p=0.0175$) on pSer31 levels in the LC in animals exposed to footshock stress (Figure 4A). Post hoc analysis, indicated that relative to FS-, FS+ displayed a significant increase in pSer31 at 20 min ($p<0.001$) and 40 min ($p<0.001$). There was a significant effect of treatment ($F=9.70$, $DFn=1$, $p=0.0040$) and time ($F=7.22$, $DFn=2$, $p=0.0028$) on pSer31 levels in the LC in animals exposed to immobilization stress (Figure 4B). Post hoc analysis, indicated that relative to IMO-, IMO+ displayed a significant increase in pSer31 at 10 min ($p<0.01$). There was a significant effect of treatment ($F=27.54$, $DFn=1$, $p<0.0001$) on pSer31 levels in the AM in animals exposed to footshock stress (Figure 4C).

Post hoc analysis, indicated that relative to FS-, FS+ displayed a significant increase in pSer31 at 10 min ($p<0.05$), 20 min ($p<0.05$) and 40 min ($p<0.01$). There was a significant effect of treatment ($F=14.81$, $DFn=1$, $p=0.006$) and time ($F=9.77$, $DFn=2$, $p=0.0005$) on pSer31 levels in the AM in animals exposed to immobilization stress (Figure 4D). Post hoc analysis, indicated that relative to IMO-, IMO+ displayed a significant increase in pSer31 at 20 min ($p<0.01$).

The effect of footshock or immobilization stress on TH phosphorylation at Ser40 in the locus coeruleus and the adrenal medulla

Rats were exposed to either footshock (Figures 5A, C) or restraint stress (Figures 5B, D) for 10, 20 or 40 mins. The LC and AM were collected and TH phosphorylation levels at Ser40 were determined. There was no effect of treatment or time on pSer40 levels in the LC or the AM in any of the groups analysed (Figures 5A, 5B, 5C and 5D). Post hoc analysis, indicated that relative to FS-, FS+ displayed a significant decrease in pSer40 in LC at 40 min ($p<0.01$) (Figure 5A).

DISCUSSION

We have investigated the effects of two different acute stressors on TH phosphorylation in rat LC and AM. The stressors used included footshock, which is primarily a physical stressor (pain) when used acutely and immobilization stress, which is primarily a psychological stressor (Dayas et al., 2001). We found that there were no changes in TH protein levels in the LC and AM over the first 40 min with either stressor. However, there were changes in TH phosphorylation at Ser19, Ser31 and Ser40 in both the LC and the AM and the responses to the two stressors were markedly different. Immobilisation stress caused only transient increases in Ser31 phosphorylation, while footshock stress both decreased Ser19 phosphorylation over time and increased Ser31 phosphorylation at all times investigated. These findings indicated that immobilisation stress had significantly less impact than footshock on stimulating the LC and AM cells and thereby activating TH.

Our data are in keeping with previous studies on the effects of immobilization and footshock stress on TH protein levels. In the LC, there was no change in TH protein levels within the 2 hr of a single immobilization stress, while there was a significant up regulation in TH protein observed 24 hr after a single immobilization stress for 2 hr (Hebert et al., 2005). In the AM, a single 2 hr immobilization stress caused a slight, but not statistically significant, rise in TH protein, while a significant up regulation in TH protein was observed 6 hr after the immobilization stress (Nankova et al., 1994). TH mRNA, but not TH protein, has been investigated in the LC after a single 15 min footshock stress; there was a delayed increase in TH mRNA level 3 hr following the end point of exposure that lasted 24 hr (Chang et al., 2000). Therefore, the earliest time that TH mRNA or TH protein can be used to detect

catecholaminergic cell activation in response to footshock or immobilization stress is 3 hr and 6 hr respectively.

Our data show that footshock stress evokes changes in TH phosphorylation at Ser19 at much earlier times than seen with any of the changes in TH mRNA or TH protein. In contrast with immobilization stress there were no treatment effects overall, nor were there treatment differences seen at any individual time point. With footshock stress the treatment significantly decreased Ser19 phosphorylation overall in both tissues; this was seen especially after 10 and 20 min in the AM and after 40 min in the LC. The decrease in Ser19 phosphorylation in response to footshock stress, relative to the FS- group, could be due to either less activation of protein kinase(s), or to a greater activation of protein phosphatases. In primary adrenal chromaffin cell cultures Ser19 is rapidly phosphorylated and then rapidly dephosphorylated (Haycock, 1993). This phosphorylation is likely to be due to the depolarization of the cells, leading to the entry of extracellular calcium via voltage sensitive calcium channels and then the direct activation of the calcium dependent kinase CaMPKII (Dunkley et al., 2004). The subsequent dephosphorylation is likely to be due to activation of protein phosphatases as a result of the continually increasing levels of calcium (Robinson and Dunkley, 1985). If calcium levels rise rapidly then rapid activation of the protein phosphatases leads to decreased phosphorylation without the initial increase being observed (Robinson and Dunkley, 1985). The fact that Ser19 phosphorylation progressively decreased in both tissues to well below that seen in the HCC suggests that greater phosphatase activation occurs in response to treatment, presumably due to greater activation of the cells and greater calcium entry. These data suggest that Ser19 phosphorylation is a very sensitive index of LC and AM activation in response to footshock, but not to immobilization.

Our data show that both footshock and immobilization stress evokes TH phosphorylation at Ser31. With footshock stress the treatment significantly increased Ser31 phosphorylation overall in both tissues; this was seen especially after 20 and 40 min in the LC and at all times in the AM. With immobilization stress the treatment significantly increased Ser31 phosphorylation overall in both tissues; however this was only seen at 10 min in the LC and 20 min in the AM. In primary adrenal chromaffin cell cultures Ser31 is always phosphorylated more slowly than Ser19 or Ser40 and Ser31 was not readily dephosphorylated over the first hour (Haycock, 1993; Bobrovskaya et al., 2007a; Bobrovskaya et al., 2007b). This is likely to be because the Ser31 kinases (ERK1/2) require a number of precursor kinases to be activated before they become activated and because the Ser31 phosphatases are not calcium sensitive and require inactivation of the kinases to allow dephosphorylation to occur (Dunkley et al., 2004). Immobilization stress increased Ser31 phosphorylation only transiently, while footshock stress increased Ser31 in a sustained manner and in the LC the increase continued to increase to approximately 4 fold by 40 min. A significant activation of ERK2 was observed after a single immobilization stress at 5 min in LC consistent with our data (Hebert et al., 2005). These results suggest that the extent of activation of the LC and AM was much less in response to immobilization than in response to footshock stress and this is consistent with the results seen with Ser19.

Our data show that footshock and immobilization stress has no significant overall effects on TH phosphorylation at Ser40. However, the FS- group was significantly larger than the FS+ group, but only at 40 min. In primary adrenal chromaffin cell cultures Ser40 is phosphorylated at an intermediate time between Ser19 and Ser31 and then only very slowly dephosphorylated over the first hour (Haycock, 1993; Bobrovskaya et al., 2007a; Bobrovskaya et al., 2007b). A large number of protein kinases can phosphorylate Ser40

(Dunkley et al., 2004). Clearly footshock and immobilization stress did not activate either the LC or the AM cells in a manner which led to Ser40 phosphorylation. Ser40 can be phosphorylated *in vivo* in response to stressors as we have recently shown that Ser40 phosphorylation is increased in the LC in response to social defeat after 10 min (Ong et al., 2011) and in the AM after 20 min in response to 2-deoxy-D-glucose (2DG) treatment (Bobrovskaya et al., 2010). The reason for these differences in response must be related to the different frequency of cell firing and activation of different signalling pathways that occur with the different stressors.

TH is the rate-limiting enzyme in catecholamine synthesis and TH phosphorylation at Ser19, Ser31 and Ser40 can lead to TH activation and an increase in catecholamine synthesis. The catecholaminergic cells synthesize new catecholamines in order to replenish those released into the circulatory system in response to stressors. We have shown here that footshock and immobilization stress lead to quite different activation of the catecholaminergic cells in the LC and AM when using TH phosphorylation at Ser19, Ser31 and Ser40 as markers of cell activation. Our results show that footshock stress activates these cells to a greater extent than immobilization stress. The effects of footshock on Ser19 phosphorylation shed no light on whether TH is activated in these cells as Ser19 phosphorylation does not activate TH directly. However, the fact that Ser31 phosphorylation was markedly increased and sustained in response to footshock would suggest that TH activity is likely to be increased in these cells as Ser31 phosphorylation increases TH activity directly. However, as there was no increase in Ser40 phosphorylation in response to these stressors then the extent of any increase in TH activity would most likely be modest. Other stressors such as social defeat (Ong et al., 2011) ([Ong et al., 2011](#)) and glucoprivation in response to 2DG ([Bobrovskaya et al., 2010](#)) clearly increase Ser40 phosphorylation (Bobrovskaya et al., 2010) and therefore TH activity in these

tissues. Therefore, footshock stress and especially immobilization could be considered relatively mild stressors as they did not require substantial activation of TH to replace secreted catecholamines.

CONCLUSION

Measuring TH phosphorylation at Ser19, Ser31 and Ser40 over time can be used as a sensitive index to differentiate the effects of different stressors on catecholaminergic cells. This approach has been used to show that immobilization stress is a substantially milder stressor of catecholaminergic systems than footshock stress.

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Figure Legends:

FIGURE 1: Representative immunoblots show the effect of 20 min footshock stress (FS) or 20 min immobilization stress (IMO) on the levels of total-TH protein, β -actin and phospho-TH (pSer19, pSer31 and pSer40) in the AM.

FIGURE 2: The regulation of total-TH protein (relative to β -actin), in the locus coeruleus (LC) and the adrenal medulla (AM), 10, 20 and 40 min after the treatment with footshock stress (FS) (n=6 for each time point) or immobilization stress (IMO) (n=6 for each time point). The results are presented relative to the home cage control (HCC) animals.

FIGURE 3: The regulation of TH phosphorylation at Ser19 (relative to total-TH), in the locus coeruleus (LC) and the adrenal medulla (AM), 10, 20 and 40 min after the treatment with footshock stress (FS) (n=6 for each time point) or immobilization stress (IMO) (n=6 for each time point). The results are presented relative to the home cage control (HCC) animals. *p<0.05, **p<0.01, ***p<0.001.

FIGURE 4: The regulation of TH phosphorylation at Ser31 (relative to total-TH), in the locus coeruleus (LC) and the adrenal medulla (AM), 10, 20 and 40 min after the treatment of footshock stress (FS) (n=6 for each time point) or immobilization stress (IMO) (n=6 for each time point). The results are presented relative to the home cage control (HCC) animals. *p<0.05, **p<0.01, ***p<0.001.

FIGURE 5: The regulation of TH phosphorylation at Ser40 (with respect to total-TH), in the locus coeruleus (LC) and the adrenal medulla (AM), 10, 20 and 40 min after the treatment of footshock stress (FS) (n=6 for each time point) or immobilization stress (IMO) (n=6 for each time point). The results are presented relative to the home cage control (HCC) animals. **p<0.01.

Figures:

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5