PPARa modulation of mesolimbic dopamine transmission rescues depression-related behaviors

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Running title: PPARα in a chronic stress model of depression.

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ABSTRACT

Depressive disorders cause a substantial burden for the individual and the society. Key depressive symptoms can be modeled in animals and enable the development of novel therapeutic interventions. Chronic unavoidable stress disrupts rats' competence to escape noxious stimuli and self-administer sucrose, configuring a depression model characterized by escape deficit and motivational anhedonia associated to impaired dopaminergic responses to sucrose in the nucleus accumbens shell (NAcS). Repeated treatments that restore these responses also relieve behavioral symptoms. Ventral tegmental area (VTA) dopamine neurons encode reward and motivation and are implicated in the neuropathology of depressive-like behaviors. Peroxisome proliferator-activated receptors type-α (PPARα) acutely regulate VTA dopamine neuron firing via β2 subunit-containing nicotinic acetylcholine receptors (β2*nAChRs) through phosphorylation and this effect is predictive of antidepressant-like effects.

Here, by combining behavioral, electrophysiological and biochemical techniques, we studied the effects of repeated PPARα stimulation by fenofibrate on mesolimbic dopamine system. We found decreased β2*nAChRs phosphorylation levels and a switch from tonic to phasic activity of dopamine cells in the VTA, and increased phosphorylation of dopamine and cAMP-regulated phosphoprotein Mr 32,000 (DARPP-32) in the NAcS. We then investigated whether long-term fenofibrate administration to stressed rats reinstated the decreased DARPP-32 response to sucrose and whether this effect translated into antidepressant-like properties. Fenofibrate restored dopaminergic responses to appetitive stimuli, reactivity to aversive stimuli and motivation to self-administer sucrose. Overall, this study suggests PPARα as new targets for antidepressant therapies endowed with motivational anti-anhedonic properties, further supporting the role of an unbalanced mesolimbic dopamine system in pathophysiology of depressive disorders.

Keywords: depression, chronic stress, peroxisome proliferator-activated receptor alpha, dopamine neurons, nicotinic acetylcholine receptors, self-administration.

Chemical compounds studied in this article.

Fenofibrate (PubChem CID: 3339).

1. INTRODUCTION

Depression is a common illness worldwide, with an estimated 350 million people affected (Reed et al., 2015), and is one of the priority conditions included in the WHO's Mental Health Gap Action Programme. Different classes of antidepressants are available and efficacious, yet about one third of depressed patients do not adequately respond to treatment or experience significant side effects (Trivedi et al., 2006), thus highlighting the need for novel therapies. Anhedonia is a frequent symptom in depressed patients (Pelizza and Ferrari, 2009) that is predictive of poor treatment response (Wichers et al., 2012), as current antidepressants often fail to relieve this symptom (McCabe et al., 2010; Nutt et al., 2007). However, clinical diagnosis still does not clearly distinguish between different aspects of anhedonia such as a decrease in experienced pleasure or in motivation and effort-related motivational dysfunctions. On the other hand, the neurobiological mechanisms underpinning the consummatory ("liking") and preparatory ("wanting") behaviors controlled by positive stimuli allow the distinction between pleasure and motivation in experimental models (Treadway and Zald, 2011). Thus, rats can be trained to press a lever in order to self-administer sucrose and the effort they are willing to exert in order to obtain the reinforcing stimulus can be measured by the breaking point (BP) score that is considered an index of animal motivation (Salamone et al., 2012). In particular, effort-related motivational behaviors are clearly dependent on dopamine D_1 receptor transmission in the NAc (Yohn et al., 2015).

The dopamine system, arising from the ventral tegmental area (VTA) and projecting to target limbic and cortical regions, is involved in reward-related learning, motivation, decision-making processes (Wise, 2004). Studies in experimental models demonstrated that VTA dopamine neuronal activity plays a role in the pathophysiology of depressive symptoms and that its manipulation reinstates physiological responses to positive and negative stimuli (Chaudhury et al., 2013; Friedman et al., 2005; Friedman et

al., 2008; Friedman et al., 2012; Friedman et al., 2014; Li et al., 2011; Nestler and Carlezon, 2006; Russo and Nestler, 2013; Yadid and Friedman, 2008). Therefore, the modulation of VTA dopamine neuronal activity may have a great potential for the development of new antidepressant strategies. Indeed, phasic activity of VTA dopamine neurons appears essential for the response to commonly used antidepressants (Chaudhury et al., 2013; Friedman et al., 2008). In this context, the regulation of VTA dopamine neural activity by peroxisome proliferator-activated receptors type-α (PPARα) (Melis et al., 2013a; Melis et al., 2010) appears potentially relevant (Melis and Pistis, 2014) for the ensuing behavioral effects, including an antidepressant-like activity (Melis et al., 2013b). In fact, acute activation of PPARα via negative modulation of β2 subunit-containing nicotinic acetylcholine receptors (β2*nAChRs) decreases dopamine neuron firing rate and the proportion of spontaneously active neurons (Melis et al., 2010; Melis et al., 2013b). Remarkably, β2*nAChRs on VTA dopamine cell bodies are essential for the transition from tonic to phasic activity of these neurons (Mameli-Engvall et al., 2006), and phasic stimulation of VTA dopamine neurons reverses chronic stress-induced depressive-like behaviors (Tye et al., 2013). Exposure to a protocol of repeated unavoidable stress induces in rats a depressive-like phenotype by disrupting the competence to avoid aversive stimuli (escape deficit) and operate for positive stimuli in Y-maze or self-administration (SA) protocols (Gambarana et al., 2001). The expression of these behavioral deficits is associated to blunted dopaminergic responses to a positive stimulus such as sucrose. In fact, the response to sucrose consumption is associated to a dopaminergic response in the nucleus accumbens shell (NAcS) in terms of extraneuronal dopamine levels (Danielli et al., 2010) and dopamine D_1 receptor-dependent signaling (Rauggi et al., 2005; Scheggi et al., 2013). In particular, an increase in PKA-dependent phosphorylation of the Thr34 residue of dopamine and cAMP-regulated phosphoprotein Mr 32,000 (DARPP-32) is observed after sucrose consumption in the NAcS (Rauggi et al., 2005; Scheggi et al., 2013). However, in rats exposed to a chronic stress protocol, sucrose consumption fails to enhance extraneuronal

dopamine levels and phospho-Thr34 DARPP-32 levels in the NAcS (Marchese et al., 2013; Scheggi et al., 2015; Scheggi et al., 2013). Noteworthy, pharmacological treatments that restore the dopaminergic response to sucrose also reinstate sucrose operant behavior (Marchese et al., 2013; Scheggi et al., 2015; Scheggi et al., 2013).

On these bases, we hypothesized that long-term activation of PPARα by fenofibrate (FBR), a clinically available lipid-modifying drug (Fruchart et al., 1999), might modulate VTA dopamine neuronal activity *in vivo* and be effective in a rat model of stress-induced depressive-like phenotype displaying motivational anhedonia and hyporeactivity to aversive stimuli.

2. MATERIALS AND METHODS

2.1 Animals

Experiments were carried out on male Sprague Dawley rats (Harlan Laboratories, San Pietro al Natisone, Italy), weighing 200-225 g when the experimental procedures began, after a 10-day habituation to the animal colony. Rats were group-housed (4-5 animals per cage) in an environment maintained at a constant temperature and humidity with a 12 h reverse light/dark cycle (1900 hours lights on), and free access to food and water. Behavioral experiments were carried out between 0900 and 1700 h under red light and controlled noise conditions in non food-deprived rats. The procedures used were in accordance with the European legislation on the use and care of laboratory animals (EU Directive 2010/63) and were approved by the Ethics Committees of the Universities of Siena and Cagliari. All efforts were made to minimize the number of animals used and their suffering.

2.2 Immunoprecipitation

VTA, identified using the Atlas of Rat Brain corresponding to plate 44 (Paxinos and Watson, 2007), was excised using the rapid head-freeze dissection technique (Danielli et al., 2010). nAChR β2 subunit immunoprecipitation was performed as described (Melis et al., 2013b) from tissue lysate using a polyclonal anti-β2 subunit antibody (sc-11372, Santa Cruz Biotechnology, Dallas, TX, USA) coupled to protein A Dynabeads (Invitrogen, Carlsbad, CA, USA). Immunoprecipitates were analyzed by immunoblotting using an anti-phosphotyrosine antibody (Cell Signaling Technology, Beverly, MA, USA). The membranes were stripped and reprobed with anti-β2-antibody to control for equal loading. Phospho-protein levels were normalized to that of β2 subunit.

2.3 Immunoblotting

NAcS, identified using the Atlas of Rat Brain corresponding to plates 10-12 (Paxinos and Watson, 2007), was excised using the rapid head-freeze dissection technique and performed as described

(Scheggi et al., 2015). Membranes were probed with anti-phospho DARPP-32 (Thr34) and anti-DARPP-32 antibodies (Cell Signaling Technology, Beverly, MA, USA). The membranes incubated with anti-phospho DARPP-32 (Thr34) were stripped and reprobed with anti-DARPP-32 antibody to control for equal loading. Phospho-protein levels were normalized to those of DARPP-32.

2.4 Electrophysiology

Rats (63–90 d) were anesthetized with urethane (1.3 g/kg, i.p.) and placed in the stereotaxic apparatus (Kopf, Tujunga, CA, USA) with their body temperature maintained at 37 ± 1 °C by a heating pad. VTA was identified using Atlas of Rat Brain corresponding to plates 82-83 (Paxinos and Watson, 2007). Single-unit activity of neurons located in VTA (AP, −6.0 mm from bregma; lateral, 0.3–0.6 mm from midline; V, 7.0–8.0 mm from the cortical surface) was recorded extracellularly with glass micropipettes filled with 2% pontamine sky blue dissolved in 0.5 M sodium acetate (impedance, 2–5 MΩ). Single-unit activity was filtered (bandpass, 500–5000 Hz), and individual spikes were isolated by means of a window discriminator (Digitimer), displayed on a digital storage oscilloscope (TDS 3012; Tektronics). Experiments were sampled on-line and off-line with Spike2 software (Cambridge Electronic Design) by a computer connected to the CED 1401 interface (Cambridge Electronic Design). VTA dopamine neurons were selected when all criteria for identification were fulfilled: firing rate, <10 Hz; duration of action potential, >2.5 ms as measured from start to end; inhibitory responses to hindpaw pinching (Lecca et al., 2012).

2.5 Acute escape deficit induction and chronic stress protocol

The experimental procedure consisted in the induction of an escape deficit by exposure to an unavoidable stress session, as described (Gambarana et al., 2001). Rats were immobilized with a flexible wire-net and administered 80 tail shocks (1 mA x 5 s, 1 every 30 s) in 50 min and 24 h later they were exposed to a shock-escape test. The criterion for the escape deficit condition was an escape

number of 0-6 out of 30 trials.

The chronic stress protocol consisted in the induction of the escape deficit and its maintenance by repeated exposure to minor unavoidable stressors on alternate days, as described (Gambarana et al., 2001). Rats were exposed to stress sessions in the afternoon, 3-4 h after the end of SA sessions. Control rats were manipulated daily by experimenters.

2.6 Self-administration procedure

Experiments were conducted as described (Marchese et al., 2013) in operant chambers (MED Associates Inc., St. Albans, VT, USA) provided with two response levers: the active lever delivered a sucrose pellet (Bio-Serv, Frenchtown, NJ, USA) into the food receptacle, while the inactive lever produced no programmed result. Experimental events and data collection were scheduled using MED Associates software (MED Associates Inc.). Non food-deprived rats were given daily 30-min sessions (0900 - 1200 h) and had free access to food and water in the home cage before and after each session. Rats were exposed to a fixed-ratio 1 (FR1) schedule until the control group reached a criterion of 50 lever presses for 2 consecutive days, then they were switched to an FR5 schedule. When the control group reached a criterion of 40 responses, rats were switched to a progressive ratio (PR) schedule, in which the number of responses required to receive a sucrose pellet was progressively increased with a step size of 3 until 5 min had elapsed without a response (breaking point, BP). BP was defined as the number of lever presses in the final completed ratio. The criterion for appetitive motivation deficit, induced by exposure to the chronic stress protocol, was a lever-pressing rate lower than 60% of the control group rate in FR1 and FR5 schedules (Scheggi et al., 2015).

2.7 Drugs and chemicals

The fenofibrate-enriched diet was a standard rodent diet (2016 Tekland Global diet) supplemented with fenofibrate 0.2% (w/w) (Sigma Aldrich, Italy), resulting in an average intake of 250 mg/kg/day, and

was purchased from Harlan Laboratories, Italy. All chemicals were purchased from commercial sources.

2.8 Statistical analysis

Data analyses were performed using Prism (GraphPad, San Diego, CA, USA). Electrophysiological, immunoprecipitation and immunoblotting results were analyzed by Student's t-test, unless otherwise specified. SA experiments data (FR5) were analyzed by 2-way repeated measures ANOVA; BP results and escape numbers were analyzed using one-way ANOVA. Data on DARPP-32 phosphorylation levels after sucrose consumption were subjected to 2-way ANOVA. *Post-hoc* analyses were performed by the Bonferroni's test, unless otherwise specified, when $P < 0.05$.

3. RESULTS

3.1 Effects of fenofibrate on phosphorylation levels of nAChR β2 subunit and dopamine neuronal activity in the VTA, and dopamine D_1 receptor signaling in the NAcS

A 14-day exposure to FBR diet decreased β2 subunit phosphorylation levels in the VTA ($P < 0.01$; Figure 1A). To test for a causal link between the reduced β2 subunit phosphorylation levels and VTA dopamine neuron activity, we performed a sampling of the VTA by recording spontaneous activity of putative dopamine cells in both *FBR* and *Control* rats. Recorded neurons were located within the medio-lateral aspects of the parabrachial pigmented nuclei of the posterior VTA (Figure 1B). Although the VTA exhibits cellular heterogeneity (Ford, 2006; Lammel et al., 2008; Margolis et al., 2008; Yamaguchi et al., 2011), this subregion contains the largest density of dopamine neurons (Lammel et al., 2015; Yamaguchi et al., 2011). The number of spontaneously active dopamine cells was similar in *FBR* (1.7 ± 0.1, $n = 6$) and *Control* rats (1.5 ± 0.3, $n = 9$, $P > 0.05$; (Figure 1C). An analysis of baseline electrophysiological parameters revealed that the average firing rate in *FBR* rats (3.8 ± 0.2 Hz, $n = 61$) was not different from *Control* rats (3.4 ± 0.2 Hz, $n = 60$, $P > 0.05$; Figure 1D). However, the coefficient of variation (CV), an index of firing irregularity, was increased in *FBR* rats when compared to *Control* rats (*FBR*: 67.2 ± 3.6, $n = 61$; *Control*: 57.1 ± 3.1%, $n = 60$, $P < 0.05$, data not shown). Consistently, firing pattern of dopamine neurons recorded from *FBR* animals displayed a considerably high percentage of spikes in bursts (21.3 ± 3.3 %, $n = 61$; 12.4 ± 2.1%, $n = 60$, in *FBR* and *Control* rats, respectively, $P < 0.05$; Figure 1E). A more detailed analysis of burst episodes, which includes parameters as the mean spikes per burst (MSB) and the mean burst duration (MBD), unveiled that *FBR* rats displayed longer burst episodes (MBD: 153.0 ± 11.7 ms, n= 52, vs. 106.7 ± 11.1 ms, n= 51, *FBR* vs. *Control*, respectively; $P < 0.01$; Figure 2A) and included more spikes on average (MSB: 3.15 ± 0.15, n=52, vs. 2.5 ± 0.09, n=51; *FBR* versus *Control*, respectively; $P < 0.001$, Figure 2B). Inter-spike intervals (ISI) from each recording were analyzed and an ISI histogram was built for each group. As

shown in Figure 2C, the best fit value for ISI mean of *FBR* group was lower (0.14 ± 0.01 s) than that of the *Control* rats (0.17 ± 0.003 s; *FBR* versus *Control*: $F_{3,194}$=26.58, $P < 0.0001$), thus revealing that *FBR* diet increased the number of neurons with a reduced ISI. In addition, when considering firing rate and bursting activity, dopamine neurons can be divided into 4 groups (with a cutoff of burst firing set to 20% and firing rate to 4 Hz): low frequency/low burst (LFLB, firing rate < 4 Hz and burst firing < 20%); low frequency/high burst (LFHB, firing rate < 4 Hz and burst firing > 20%); high frequency/low burst (HFLB, firing rate > 4 Hz and burst firing < 20%); high frequency/high burst (HFHB, firing rate > 4 Hz and burst firing > 20%) (Mameli-Engvall et al., 2006). As shown in Figure 2D, correlation between firing rate and burst firing indicates that *FBR* rats exhibit a higher percentage of HFHB dopamine neurons than *Control* rats, thus leading to an overall difference in firing pattern distribution ($P < 0.05$; $\chi 2$ test; Figure 2d).

These results suggest an enhanced dopaminergic transmission/signaling in VTA terminal regions in *FBR* compared to *Control* rats. Hence, we measured the phosphorylation levels of Thr34 DARPP-32 in the NAcS, as an index of mesolimbic dopaminergic D_1 receptor signaling (Nishi et al., 1997). We observed increased phospho-Thr34 DARPP-32 levels in *FBR* (compared to *Control*) rats ($P < 0.05$), indicative of an increased dopaminergic D_1 receptor transmission (Figure 2E). Thus, FBR administration induces an effect that might prevent the development of and rescue from already established stress-induced deficits.

3.2 Effects of fenofibrate on the phosphorylation levels of nAChR β2 subunit and Thr34 DARPP-32 in rats exposed to chronic stress protocol

In light of the electrophysiological and neurochemical results, we hypothesized that repeated FBR administration might restore the NAcS dopamine signaling response to a positive rewarding stimulus, i.e. sucrose, disrupted by chronic stress exposure. To this end, we evaluated the DARPP-32

phosphorylation pattern at baseline and after sucrose pellet consumption, and the nAChR β2 subunit phosphorylation levels in the VTA. Rats were divided into *Control* (*n* = 18) and *Chronic Stress* (*n* = 18) groups. Rats in the latter group were exposed to the sequence of unavoidable stress and escape test (days 1 - 2) and then to the stress protocol until the end of the experiment. Beginning on day 21, two groups of rats (n=8) were fed the standard diet (*Control* and *Chronic Stress* groups) while a third one (n=10) received the FBR diet (*FBR* and *Chronic Stress + FBR* groups). After a 14-day treatment, one half of the animals was sacrificed at baseline and the other half 30 min after sucrose consumption (Figure 3A). In the VTA, analysis of β2 subunit phosphorylation levels with treatment and stress as independent variables only revealed a treatment effect, as FBR administration reduced phospho-β2 subunit levels irrespective of stress exposure ($F_{1,14}$= 15.65, $P < 0.01$; *post hoc:* $P < 0.05$, *FBR* group versus *Control* group and *Chronic Stress + FBR* group versus *Chronic Stress* group; Figure 3B, C). In the NAcS, the analysis of phospho-Thr34 DARPP-32 levels showed a treatment effect ($F_{1,14}$ = 12.65, $P < 0.01$; *post hoc*: $P < 0.05$, *FBR* group versus *Control* group; Figure 3C, D). While sucrose consumption did not modify phospho-β2 subunit levels in the VTA (data not shown), analysis of phospho-Thr34 DARPP-32 levels in the NAcS disclosed an effect of group ($F_{3,16}$ = 8.68, $P < 0.01$), time ($F_{1,16}$ = 28.33, $P < 0.001$), and their interaction ($F_{3,16}$ = 8.68, $P < 0.01$). In fact, FBR administration restored the response to sucrose, impaired by stress exposure (*post hoc*: $P < 0.001$, *Control*, *FBR* and *Chronic Stress + FBR* groups versus *Chronic Stress* group; Figure 3E). Thus, repeated FBR administration rescued D_1 receptor-dependent PKA signaling response to a natural reward in rats exposed to chronic unavoidable stress.

3.3 Administration of fenofibrate rescued from stress-induced deficits in appetitive motivation and reactivity to aversive stimuli

Since treatments that restore dopaminergic responses to sucrose in NAcS also rescue rats from motivational anhedonia reinstating stress-impaired sucrose SA (Marchese et al., 2013; Scheggi et al., 2015), we investigated whether FBR administration reversed two depressive-like symptoms in rats exposed to chronic unavoidable stress, sucrose SA and escape deficit.

A preliminary experiment was carried out to test the effects of FBR diet on the escape test and sucrose SA protocol in rats not exposed to chronic stress. A 14-day pre-exposure to FBR diet did not modify by itself the reactivity to avoidable aversive stimuli in rats, while it increased reactivity in rats exposed to an acute unavoidable stress ($F_{3,26}$ = 63,21, P < 0.001; Bonferroni's test: P < 0.001, *Naïve*, *FBR*, and *FBR + Stress* groups versus *Stress* group, Table 1). Moreover, an exposure to FBR diet before and during the SA protocol did not modify the responses to sucrose in FR1, FR5, and PR schedules, compared to standard diet-fed rats, as only training (session) affected the performance (two-way ANOVA, FR1: treatment $F_{1,50}$ = 4.19, P > 0.05; session $F_{5,50}$ = 50.83, P < 0.001; FR5: treatment $F_{1,50}$ = 0.97, , P > 0.05; session $F_{4,50}$ = 2.79, P < 0.05; PR, Student's t test = 0.61; Fig. 4A, B, C).

Rats, divided into a control group (*Control*, n = 5) and a group exposed to the sequence of unavoidable stress and escape test (days 1 - 2) and chronic stress protocol (*Chronic Stress*, n = 13), were exposed to FR1 and then FR5 schedules (Figure 5A). When the criterion for appetitive motivation deficit was attained by the *Chronic Stress* group (FR1, 5th session, correct responses: *Control*: 73.61 ± 6.0, *Chronic Stress*: 30.81 ± 1.9, P < 0.001; FR5, 3rd session, correct responses: *Control*: 43.20 ± 3.8, *Chronic Stress*: 12.61 ± 1.8, P < 0.001), the animals were divided into two subgroups that continued stress exposure: one group was fed the standard diet (*Chronic Stress*, n = 5) and the other the FBR diet *(Chronic Stress + FBR*, n = 8) (day 21). After 10 days of treatment (day 31), SA sessions were resumed under a FR5 schedule for 6 sessions (Figure 5A). Analysis of lever press numbers revealed an effect of group ($F_{2,90}$ = 42.16, P < 0.001) and session ($F_{5,90}$ = 2.85, P < 0.05). In particular, performance was reduced by stress exposure (*Chronic Stress* versus *Control* group, P < 0.01, session 6), while FBR

administration restored the performance in *Chronic Stress + FBR* group at *Control* group level (*Chronic Stress + FBR* versus *Chronic Stress* group, $P < 0.01$, sessions 2-4; $P < 0.001$, sessions 5 and 6; Figure 5B). Analysis of breaking point (BP) values showed that stress exposure reduced the motivation to lever press for sucrose, and FBR diet rescued incentive motivation in rats exposed to chronic stress ($F_{2,15} = 11.85$, $P < 0.001$; *post hoc*: $P < 0.05$, *Chronic Stress* versus *Control* group, $P < 0.001$, *Chronic Stress + FBR* versus *Chronic Stress* group; Figure 5C). Twenty-four h after the last SA session, rats were exposed to the escape test to assess their reactivity to avoidable aversive stimuli. FBR diet restored the escape competence impaired by the exposure to the chronic stress protocol ($F_{2,15} =14.53$, $P < 0.001$; *post hoc*: *Chronic Stress* group, $P < 0.001$ versus *Control* group, and $P < 0.01$ versus *Chronic Stress + FBR* group; *Chronic Stress + FBR* group versus *Control* group, $P > 0.05$; Figure 5D). Altogether, these results show that long-term FBR administration is endowed with antidepressant-like activity, likely related to chronic activation of PPARα.

4. DISCUSSION

Here, we have shown that chronic activation of PPARα by a FBR enriched-diet prevented and relieved stress-induced depression-related behaviors through phasic activation of mesolimbic dopamine system. Specifically, we found that repeated activation of PPARα induced a decreased phosphorylation of β2*nAChRs that was accompanied by a switch in firing pattern of spontaneously active dopamine cells, consistent with β2*nAChRs role in gating different states of dopamine neuronal activity (Mameli-Engvall et al., 2006). In addition, we observed that PPARα activation enhanced dopamine D_1 receptor signaling in the NAcS, as indicated by the increased phospho-Thr34 DARPP-32 levels. Finally, long-term PPARα stimulation restored dopamine signaling to sucrose consumption in the NAcS, and the appetitive motivation and reactivity to aversive stimuli impaired by chronic stress exposure.

These findings are in agreement with the causal role of VTA dopamine cell activity and dopaminergic transmission in the NAcS in depressive-like symptoms produced by chronic mild stress in mice (Tye et al., 2013). They also uphold earlier findings carried out in a rat model of depression exhibiting a reduced phasic activity of VTA dopamine cells (Friedman et al., 2005) that was increased after antidepressant treatment (Friedman et al., 2008). Moreover, the present study supports and extends previous reports that suggested that PPARα activation may be endowed with antidepressant-like activity in the forced swimming test (Melis et al., 2013b; Yu et al., 2011), and in the control of depressive symptoms, as observed in mice after repeated administration of natural agonists (Jin et al., 2015; Yu et al., 2011).

In addition, the observations that chronic PPARα activation decreased β2 nAChR subunit phosphorylation in the VTA, increased burst duration and number of spikes per burst of dopamine neurons and enhanced dopamine D_1 receptor signaling in NAcS are in agreement with the established significant interaction between PPARα and β2*nAChRs in VTA dopamine cells (Melis et al., 2013a;

Melis et al., 2010; Melis et al., 2013b) and the role of mesolimbic dopamine system in depression-related behaviors (Nestler and Carlezon, 2006; Nunes et al., 2013a; Nunes et al., 2013b; Russo and Nestler, 2013). Whether or not these PPARα-dependent effects on dopamine neurons are mediated by an increase in the number of functionally responsive β2*nAChRs in the plasma membrane or in their ionic conductance is still elusive. Nonetheless, our findings indicate that long-term PPARα stimulation potentiates the effects of endogenous cholinergic transmission mediated by β2*nAChRs on the excitability of dopamine neurons by allowing them to access diverse firing patterns, i.e. high frequency/high burst firing pattern, which should favor enhanced dopamine output or phasic activity in response to relevant stimuli in terminal regions. Indeed, FBR administration restored the dopaminergic response to sucrose in the NAcS of stressed rats.

Previous findings showed that phasic optogenetic activation of VTA dopamine neurons relieved chronic stress-induced depression-like behaviors within seconds, a phenomenon that requires functioning of dopamine receptors in the NAc (Tye et al., 2013). In this study, by exposing rats to chronic stress we modeled two depressive symptoms, hyporeactivity towards negative stimuli and motivational anhedonia, which are associated with decreased dopamine D_1 receptor signaling in response to hedonic stimuli (Scheggi et al., 2015). Long-term FBR administration completely reversed these stress-induced behavioral and neurochemical deficits. Notably, we previously observed a similar effect on this model only following lithium treatment (Marchese et al., 2013), as the antidepressants imipramine and fluoxetine restore the reactivity toward aversive stimuli, but lack the effect on motivational anhedonia completely (fluoxetine) or in half of the treated rats (imipramine) in the time-frame of our experimental protocols (Scheggi et al., 2015). In clinical studies, early improvement in positive affect is predictive of antidepressant treatment response (Geschwind et al., 2011; Wichers et al., 2009). Our present and previous findings on the role of dopaminergic transmission in the motivation to operate for positive stimuli (Marchese et al., 2013; Scheggi et al., 2015) are in agreement

with recent studies indicating that pharmacological manipulations that strengthen dopaminergic transmission in the NAc, and consequently increase DARPP-32 phosphorylation levels, are able to enhance effort-related processes in rats (Nunes et al., 2013a; Randall et al., 2015; Randall et al., 2012; Salamone et al., 2016a; Salamone et al., 2016b).

Our findings not only broaden the already wide variety of PPARα functions, but also of their potential therapeutic and clinical applications in other neuropsychiatric disorders (Melis and Pistis, 2014). Hence, while they were mainly known to regulate nutrient metabolism and energy homeostasis (Grygiel-Gorniak, 2014), their important role in modulating CNS functions has emerged (Fidaleo et al., 2014; Pistis and Melis, 2010; Roy and Pahan, 2015). In this context, we add to our expanding knowledge of the neuroanatomic and physiopathological underpinnings of depressive states this nuclear receptor. Accordingly, increased plasma levels of natural PPARα ligands in humans following acute exercise might be involved in mediating its beneficial antidepressant effects (Heyman et al., 2012). Indeed, preclinical evidence suggests that boosting endogenous PPARα agonists by either preventing their degradation or increasing their synthesis yields antidepressant-like activity (Adamczyk et al., 2008; Bortolato et al., 2007; Gobbi et al., 2005; Melis et al., 2013b; Umathe et al., 2011). Hence, while we acknowledge that our study is far from pinpointing the precise pathway dysfunctions mediating the diverse symptoms of depression in experimental models, we suggest that long-term PPARα stimulation through phasic activation of mesolimbic dopamine transmission is sufficient to reinstate physiological responses to natural rewarding and aversive stimuli, and therefore propose PPARα agonists for their novel antidepressant- and motivational antianhedonic-like properties.

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Figure legends

Figure 1

Fenofibrate decreased phosphorylation levels of nAChR β2 subunit and enhanced bursting activity in VTA dopamine neurons.

(A) Rats fed standard or 0.2% fenofibrate diet (Control and FBR) for 14 days were sacrificed, VTA was excised and immunoprecipitated. Data are expressed as mean ± S.E.M. of percentage modifications in β2 subunit phosphorylation levels compared to levels in the Control group. ** $P < 0.01$, Student's t test. (B) The schematic diagram illustrates the recording protocol used for in vivo electrophysiological experiments and an example of the typical broad spike waveform of a dopamine neuron. The diagrams below show localizations of recording sites in FBR and Control rats (black dots) as verified by histological sections. On the right, the panel displays an example of a recording location for a VTA dopamine neuron in a FBR treated rat (the triangle indicates the pontamine sky blue dye). Abbreviations: RN, red nucleus; IP, interpeduncular nucleus, SN, substantia nigra pars reticulata. Scale bar, 0.5 mm. (C) The bar graph shows the mean number of spontaneously active dopamine cells in the VTA as encountered by the electrode in Control and FBR rats. (D) Traces on the left illustrate representative extracellular recordings of putative VTA dopamine neurons from Control (above) or FBR (below) rats. The scatter plot on the right displays dopamine cells firing rate in Control and FBR rats. (E) Effect of standard or 0.2% fenofibrate diet on bursting activity of VTA dopamine cells expressed as the percentage of spikes in burst. Dopamine neurons recorded from FBR rats show increased bursting when compared with Controls. * $P < 0.05$, Student's t test

Figure 2

Fenofibrate changed firing pattern of VTA dopamine neurons and dopamine D_1 receptor signaling in the NAcS.

Analysis of firing pattern of VTA dopamine neurons recorded from rats fed standard or 0.2% fenofibrate diet (Control and FBR) for 14 days yields increased mean burst duration (A), mean spikes per burst (B) and decreased inter-spike intervals (ISI) from each VTA dopamine neuron recorded (C). (D) Dopamine cells are divided into four groups depending on firing rate and burst firing: low firing low burst (LFLB), low firing high bursts (LFHB), high firing low bursts (HFLB) and high firing high bursts (HFHB). FBR rats showed a higher number of HFHB cells when compared to controls. * P<0.05 chi-squared test) (e) Phospho-Thr34 DARPP-32 levels in the NAcS of rats fed standard or 0.2% fenofibrate diet for 14 days. Data are expressed as mean ± S.E.M. of percentage modifications in Thr34 DARPP-32 phosphorylation levels compared to levels in the Control group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Student's t test.

Figure 3.

Fenofibrate modified the phosphorylation levels of nAChR β2 subunit and Thr34 DARPP-32 in rats exposed to the chronic stress protocol and restored the response to sucrose impaired by stress exposure in the NAcS.

Rats in the Chronic Stress group were exposed to the stress protocol for 3 weeks. On day 21, part of the Control and Chronic Stress rats continued to receive the standard diet (Control and Chronic Stress), while the remaining rats were fed the 0.2% fenofibrate-enriched diet (FBR and Chronic Stress + FBR) (A). After a 14-day diet exposure, animals in each group were sacrificed at baseline (B, D) or 30 min after sucrose consumption (E). (B, D) Data are expressed as mean ± S.E.M. of percentage modification in phospho-β2 subunit and phospho-Thr34 DARPP-32 levels compared to Control group levels. * $P <$ 0.05 compared to Control group; $^{\#}$ $P < 0.05$ compared to Chronic Stress group (Bonferroni's test). (C)

Representative immunoblots. (E) Data are expressed as mean ± S.E.M. of percentage modification in phospho-Thr34 DARPP-32 levels compared to levels in non sucrose-exposed groups. §§§ $P < 0.001$ compared to all other groups; ** $P < 0.01$ compared to Chronic Stress group (Dunnett's test).

Figure 4

Fenofibrate did not modify appetitive motivation for sucrose in rats not exposed to chronic stress protocol.

Rats fed the standard or 0.2% fenofibrate diet (Control, $n = 6$; FBR, $n = 6$) for 10 days were trained to lever press for sucrose pellets under FR1 (A), FR5 (B), and PR (C) schedules of reinforcement while continuing to receive the standard or 0.2% fenofibrate diet. Data are presented as the mean ± S.E.M. of the number of responses.

Figure 5

Fenofibrate reversed stress-induced deficits in appetitive motivation and reactivity to noxious stimuli.

Rats were exposed to the chronic stress and SA protocols and when the condition of motivational anhedonia was established, they continued to receive the standard diet (Chronic Stress) or began the 0.2% fenofibrate diet (Chronic Stress + FBR) (A). At day 15, they resumed the FR5 (B) and PR schedules (C), while continuing diet and chronic stress exposure (A). Data are presented as mean ± S.E.M. of the number of responses. ** $P < 0.01$ Control compared to Chronic Stress group; ## $P < 0.01$, ### $P < 0.001$ Chronic Stress + FBR compared to Chronic Stress group (Bonferroni's test). (D) Twenty-four h after the last PR session, rats were tested for escape. Scores are expressed as mean number of

escapes ± S.E.M. in 30 consecutive trials.* $P< 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared to Chronic Stress group.

Table 1. Fenofibrate prevented the development of acute escape deficit

Group	Experimental protocol	Number of escapes
Naive (*n* =5)	Standard Diet and Escape Test	21.8 ± 1.5
Stress **(***n* = 10)	Standard Diet and Unavoidable stress + Escape test	3.5 ± 1.2 ***
FBR *(n* = 5)	Fenofibrate and Escape test	26.0 ± 0.8
FBR + Stress (*n* =10)	Fenofibrate and Unavoidable stress + Escape test	22.7 ± 1.3

Rats fed the standard or fenofibrate enriched-diet for 14 days were exposed (Stress and FBR + Stress groups) or not to the unavoidable stress session (Naive and FBR). Twenty-four hours later, rats were tested for the escape. Data are expressed as mean ± S.E.M of the number of escapes/30 trials. ****P* < 0.001 compared to Naive, FBR and FBR+ Stress groups (Bonferroni's test).

FIGURE 1

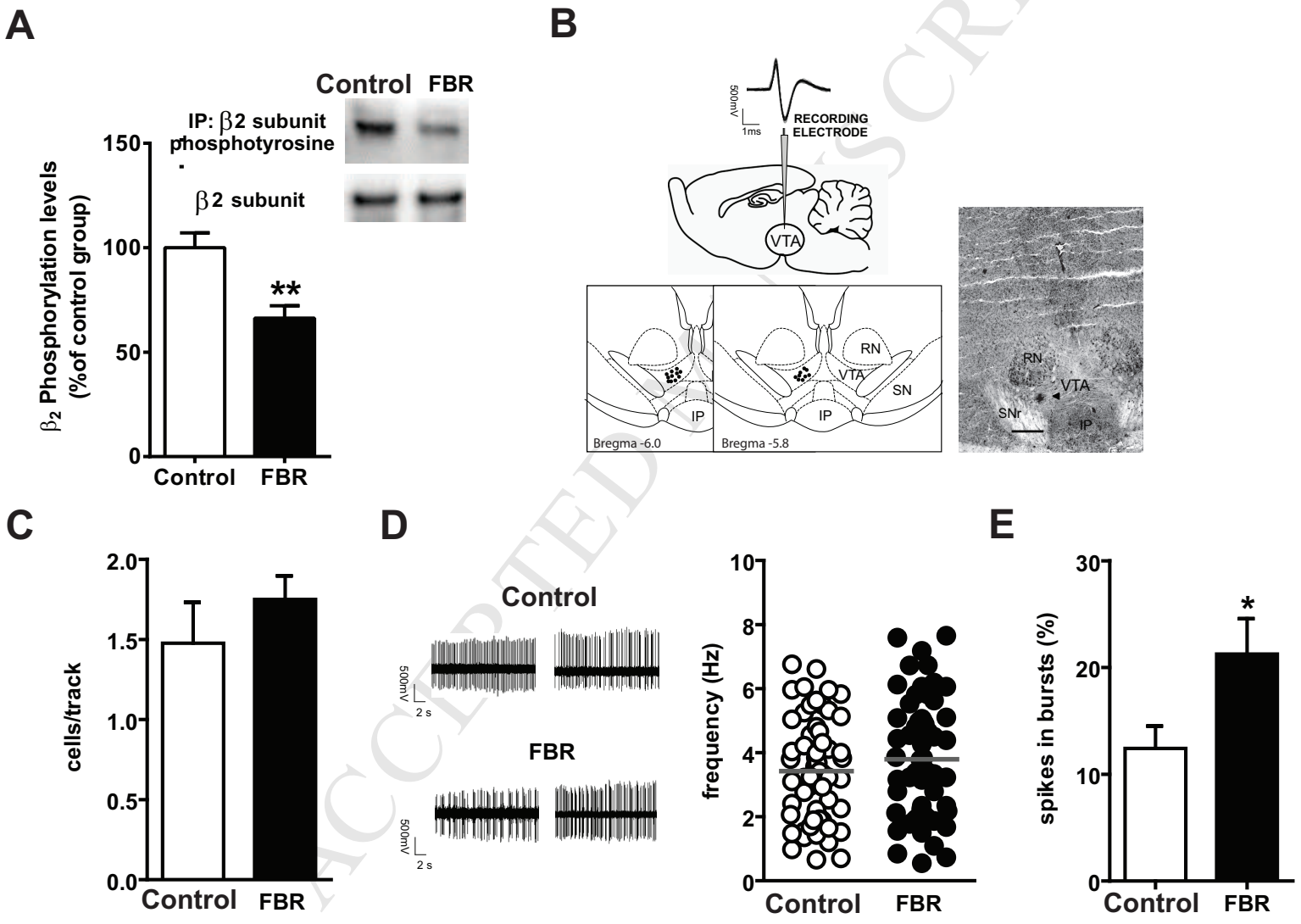

A
Control
FBR
IP: β2 subunit phosphotyrosine
β2 subunit
β2 Phosphorylation levels (%of control group)
150
100
50
0
**
Control
FBR
B
500mV
1ms
RECORDING ELECTRODE
VTA
RN
VTA
SN
IP
IP
Bregma -6.0
Bregma -5.8
RN
VTA
SNr
IP
C
cells/track
2.0
1.5
1.0
0.5
0.0
Control
FBR
D
Control
500mV
2 s
FBR
500mV
2 s
frequency (Hz)
10
8
6
4
2
0
Control
FBR
E
spikes in bursts (%)
30
20
10
0
*
Control
FBR

FIGURE 2

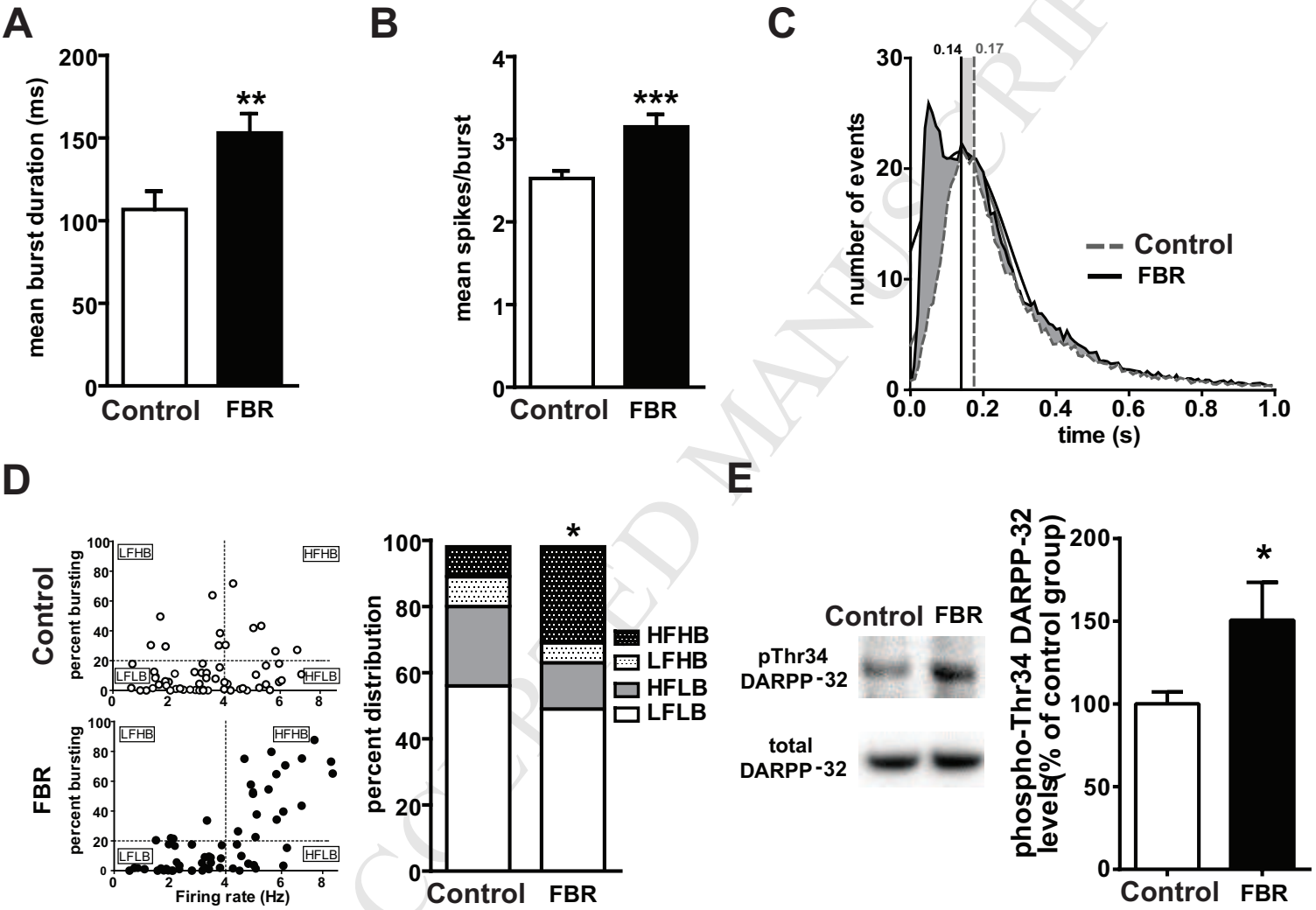

A
mean burst duration (ms)
200
150
100
50
0
**
Control
FBR
B
mean spikes/burst
4
3
2
1
0
***
Control
FBR
C
number of events
30
20
10
0
0.14
0.17
0.0
0.2
0.4
0.6
0.8
1.0
time (s)
Control
FBR
D
Control
FBR
percent bursting
LFHB
HFHB
LFLB
HFLB
0
2
4
6
8
Firing rate (Hz)
percent distribution
100
80
60
40
20
0
*
HFHB
LFHB
HFLB
LFLB
Control
FBR
E
Control
FBR
pThr34 DARPP-32
total DARPP-32
phospho-Thr34 DARPP-32 levels (% of control group)
200
150
100
50
0
*
Control
FBR

FIGURE 3

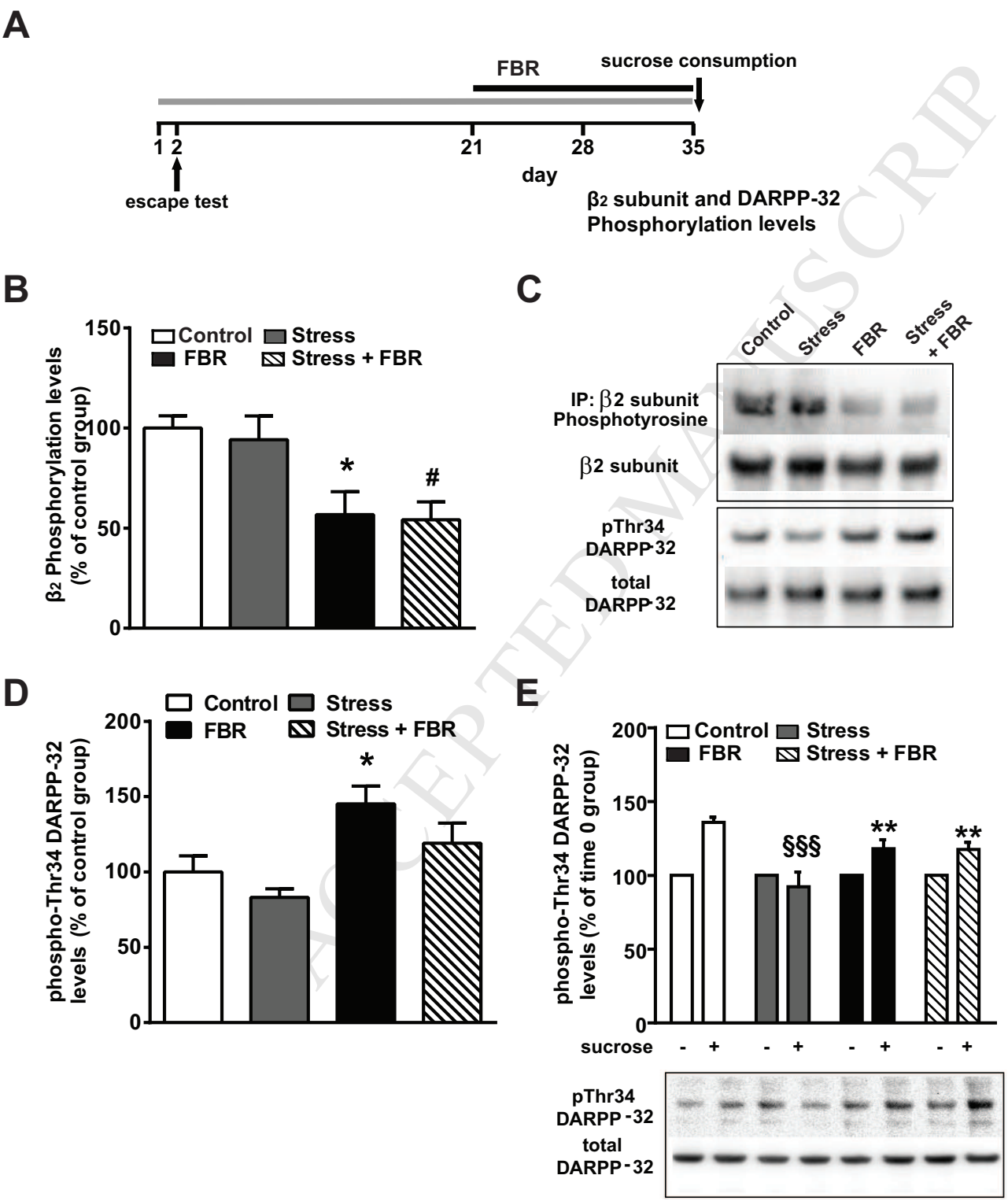

A
FBR
sucrose consumption
1 2
21
28
35
day
escape test
β2 subunit and DARPP-32
Phosphorylation levels
B
Control
Stress
FBR
Stress + FBR
β2 Phosphorylation levels
(% of control group)
150
100
50
0
*
#
C
Control
Stress
FBR
Stress
+FBR
IP: β2 subunit
Phosphotyrosine
β2 subunit
pThr34
DARPP-32
total
DARPP-32
D
Control
Stress
FBR
Stress + FBR
phospho-Thr34 DARPP-32
levels (% of control group)
200
150
100
50
0
*
E
Control
Stress
FBR
Stress + FBR
phospho-Thr34 DARPP-32
levels (% of time 0 group)
200
150
100
50
0
§§§
**
**
sucrose
- + - + - + - +
pThr34
DARPP-32
total
DARPP-32

Figure 4

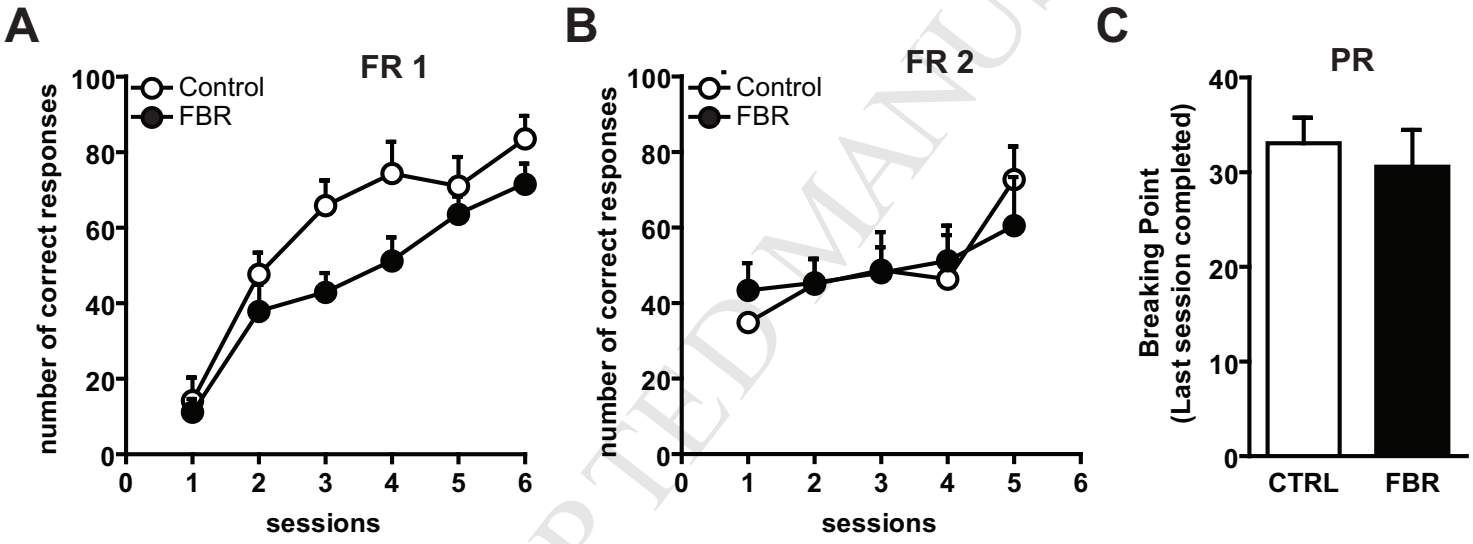
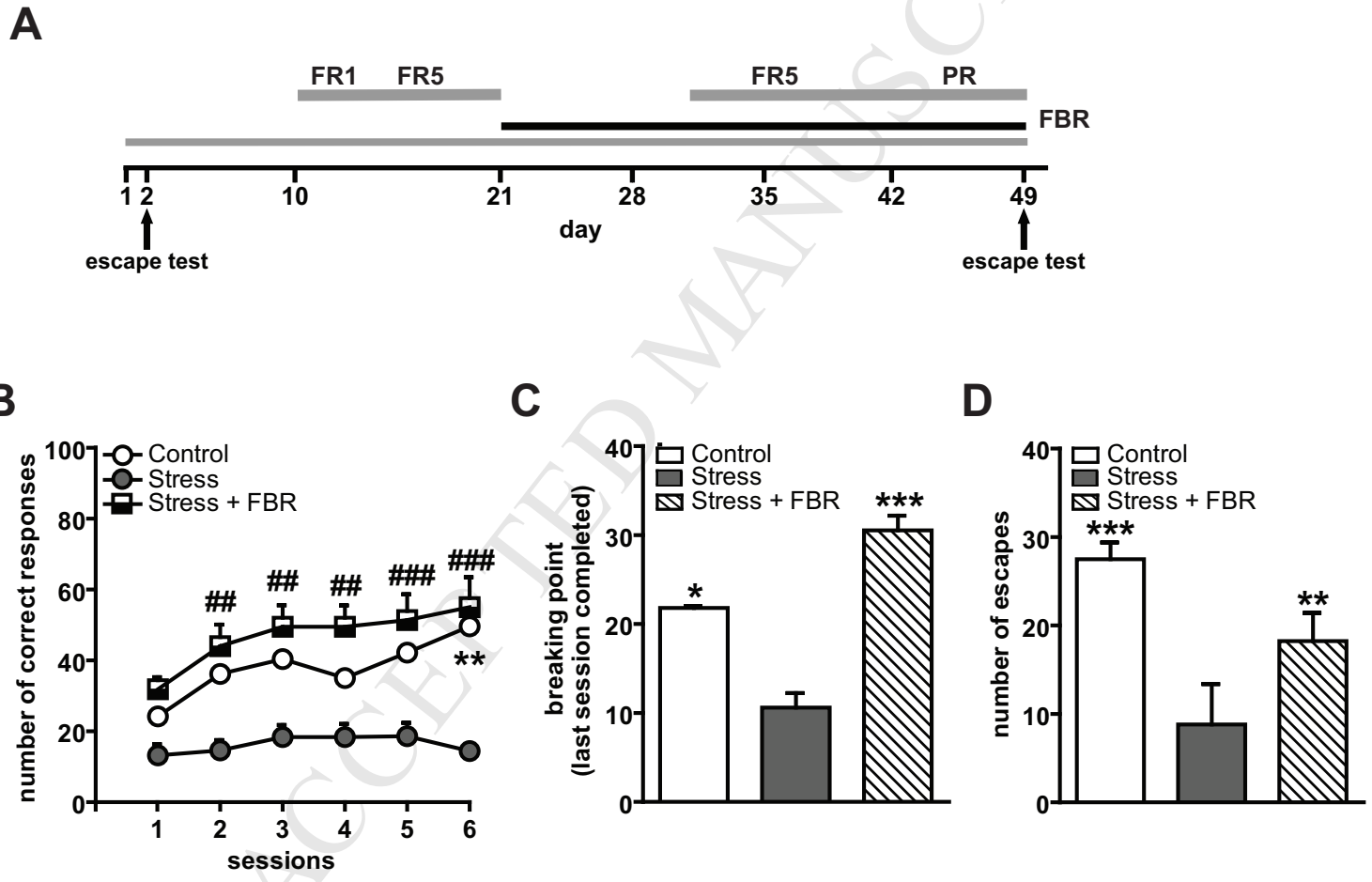


Figure 5



Highlights

- Chronic unavoidable stress induces depressive-like symptoms in rats.
- PPARα regulates dopamine transmission and is proposed to have antidepressant actions.
- Fenofibrate, a PPARα agonist, relieves stress-induced depressive phenotype.
- Fenofibrate restores the dopaminergic response to appetitive and aversive stimuli
- PPARα is a new target for antidepressant therapies.