

**Functional status of somatodendritic 5-HT_{1A} autoreceptor after chronic treatment with
fluoxetine in a mouse model of anxiety/depression based on repeated corticosterone
administration**

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Running title:

5-HT_{1A} autoreceptor sensitivity in anxio-depressive mice

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Abbreviations

5-HT, serotonin; DR, dorsal raphe; GR, glucocorticoid receptor; GRE, glucocorticoid receptor responsive element; HPA, hypothalamic-pituitary-adrenocortical; OCT, organic cation transporter; SSRI, serotonin selective reuptake inhibitor; TpH, tryptophane hydroxylase; UCMS, unpredictable chronic mild stress.

Abstract

Most of preclinical studies investigating the effects and the mechanism of action of antidepressants have been performed in naïve rodents. This is inappropriate since antidepressants act on specific symptoms of the pathology such as distress and anxiety. Recently, we have developed a mouse model of anxiety/depression based on corticosterone addition in the drinking water. This model is highly reproducible and easy to set up compared to the unpredictable chronic mild stress. 5-HT_{1A} autoreceptor is known to play a role in mood disorders and their treatments. An increase in somatodendritic 5-HT_{1A} autoreceptor density in the dorsal raphe (DR) attenuates the therapeutic activity of selective serotonin reuptake inhibitors (SSRIs), whereas their functional desensitization promotes activation of brain serotonergic transmission, thereby representing an adaptive change relevant to their therapeutic effect. Here we assessed the effects of sustained administration of the SSRI fluoxetine on 5-HT_{1A} autoreceptor sensitivity in mice administered with corticosterone. Fluoxetine attenuated the 5-HT_{1A} receptor agonist 8-Hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT)-induced hypothermia, decrease in DR 5-HT neuronal activity and 5-HT release in both vehicle- and corticosterone-pre-treated mice. However, such desensitization was more pronounced in corticosterone pre-treated mice. This change had an overall effect on serotonergic tone since we found a greater firing rate of 5-HT neurons associated with an enhancement of 5-HT outflow in the DR of corticosterone-pre-treated mice in response to fluoxetine compared to the corresponding group of vehicle-pre-treated mice. These results provide cellular explanations on the reasons why SSRIs produce antidepressant effects in pathological conditions but not in naïve animals or healthy volunteers.

Introduction

Alterations in multiple biological markers are implicated in the neurobiology of depression, based primarily on the characterization of antidepressant efficacy in naive rodents (Gourley and Taylor, 2009). However it appears more appropriate to perform pharmacological studies in animal models that exhibit hallmark characteristics of anxiety/depression. Several animal models have been developed, mainly based on stressful situations such as unpredictable chronic mild stress (UCMS). Although UCMS has been efficiently used to assess antidepressant activity (Farley et al., 2010; Surget et al., 2009), it is notoriously difficult to reproduce consistently in rodents. An intriguing alternative may be to supply mice with exogenous corticosterone (David et al., 2009; Gourley and Taylor, 2009), a hormone produced in the adrenal glands in response to stress, and found to be elevated in several animal models of depression and in depressed humans (Sterner and Kalynchuk, 2010). We have recently reported some behavioral abnormalities in mice administered with corticosterone indicative of anhedonia and hopelessness (David et al., 2009) that mimic depressive symptoms observed in humans (Holsboer, 2000; Nemeroff and Valle, 2005). Therefore corticosterone-treated mice provide a good preclinical model to investigate the interaction between hypothalamic-pituitary-adrenocortical (HPA) axis dysfunction and antidepressant response.

The activity of the serotonergic system is regulated by several extrinsic and intrinsic factors. In the dorsal raphe (DR), there is a negative feedback control driven by serotonin-1A (5-HT_{1A}) autoreceptor located in the soma and dendrites of 5-HT neurons. Activation of this presynaptic autoreceptor inhibits the firing rate of 5-HT neurons, the amount of 5-HT released per action potential, and 5-HT synthesis (Blier and de Montigny, 1987; Richardson-Jones et al., 2010). Clinical and preclinical studies have clearly established the role of this autoreceptor in mood disorders and their treatments. For example, enhanced radioligand binding of an

agonist to the inhibitory 5-HT_{1A} autoreceptor in the human DR provided pharmacological evidence of diminished activity of serotonergic neurons in suicide victims afflicted with major depression (Stockmeier et al., 1998). In addition, a functional polymorphism in the promoter region of the human Htr1a gene was reported (Lemondé et al., 2003) suggesting that an increase in the density of 5-HT_{1A} autoreceptor in the DR may predispose to depression (Le Francois et al., 2008; Lemondé et al., 2003). The role of 5-HT_{1A} autoreceptor in the mechanism of action of serotonin selective reuptake inhibitors (SSRIs) has also been studied extensively. It is believed that an over-activation and/or -expression of 5-HT_{1A} autoreceptor would delay the onset of antidepressant effect, whereas the functional desensitization of this receptor after sustained administration of SSRIs is an adaptive change relevant to their therapeutic activity (Gardier et al., 1996). Using a new strategy to manipulate somatodendritic 5-HT_{1A} autoreceptor in raphe nuclei without affecting 5-HT_{1A} heteroreceptor, it has been shown that mice with a low expression of autoreceptor display a greater behavioral response to fluoxetine, thus establishing a causal relationship between 5-HT_{1A} autoreceptor levels and the response to SSRIs (Richardson-Jones et al., 2010).

Surprisingly, despite the importance of somatodendritic 5-HT_{1A} autoreceptor desensitization in the appearance of the therapeutic effects of SSRIs, such functional inactivation was also reported in several animal models of stress including chronic sleep restriction (Evrard et al., 2006; Novati et al., 2008) and maternal separation (Van Riel et al., 2004), but also in animal models of anxiety/depression i.e., the chronic mild stress (Bambico et al., 2009; Froger et al., 2004; Grippo et al., 2005; Lanfumey et al., 1999). These results are consistent with findings showing an attenuation of 5-HT_{1A} receptor functions in a dysregulated HPA axis in both animals (Fairchild et al., 2003; Hensler et al., 2007; Lanfumey et al., 1999; Leitch et al., 2003) and humans (McAllister-Williams et al., 2007; Young et al., 1994). However, these results in animal models of stress have been challenged by recent findings showing, on the contrary,

that the sensitivity and/or the density 5-HT_{1A} autoreceptor in the DR was increased in rats or mice models of depression (El Yacoubi et al., 2003; Greenwood et al., 2003; Pineda et al., 2011). Thus modeling stress or depression status in animals leads to distinct changes in 5-HT_{1A} autoreceptor sensitivity.

The present study was aimed at determining the nature and intensity of changes in 5-HT_{1A} autoreceptor function in a mice model of anxiety/depression exposed to chronic corticosterone given either alone or in combination with the SSRI fluoxetine. Ultimately, such data obtained in an animal model of anxiety/depression may help shed light on the fact that studying the mechanism of action of SSRIs is more relevant in a disease state.

Material and Methods

Animals.

Adult male C57BL/6J mice were purchased from Elevage Janvier (Le Genest St. Isle, France). All corticosterone-administered mice were 7-8 weeks old and weighed 20-24 g at the beginning of the treatment. They were maintained on a 12L:12D schedule (lights on at 06:00 a.m.) and housed in groups of five. Food and water were provided *ad libitum*. Behavioral testing occurred during the light phase between 08:00 a.m. and 05:00 p.m. Separated groups were used to assess the behavioral, electrophysiological and neurochemical studies. All testing were conducted in compliance with protocols approved by the Institutional Animal Care and Use Committee (Council directive #87-848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale, permissions #92-256B to D.J. David).

Drugs.

In place of normal drinking water, grouped-housed mice were presented during 7 weeks with vehicle (0.45% hydroxypropyl- β -cyclodextrin) or corticosterone (35 μ g/ml) in the presence or absence of the SSRI fluoxetine (18 mg/kg/day) during the last three weeks of the corticosterone regimen. 8-Hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT) hydrobromide and (N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl)cyclohexanecarboxamide tri-hydrochloride (WAY100635) were obtained from Sigma-Aldrich (L'Isle d'Abeau, France) and dissolved in saline solution (NaCl, 0.9%). Both pharmacological compounds were administered subcutaneously (s.c.) at the doses of 100-300 μ g/kg and 300 μ g/kg; respectively.

Body temperature.

Body temperature was assessed intrarectally, using a lubricated probe (BIO-BRET-3) inserted approximately 2 cm and monitored (BIO-TK9882, BIOSEB, Vitrolles, France). Three baseline body temperature measurements were taken to control that stress induces hyperthermia (Supplemental table) according to Van der Heyden et al., (1997). Ten minutes after the third baseline measurement, animals received 8-OHDPAT (100 μ g/kg; s.c.) and body temperature was measured 10 min after the injection.

In vivo electrophysiology.

Mice were anesthetized with chloral hydrate (400 mg/kg; i.p) and placed in a stereotaxic frame with the skull positioned horizontally. To maintain a full anesthetic, chloral hydrate intraperitoneal supplements of 100 mg/kg were given as needed. The extracellular recordings were carried out using single glass micropipettes (Stoelting Europe, Dublin, Ireland) for recordings in the DR. Micropipettes were preloaded with fiberglass strands to promote capillary filling with a 2 M NaCl solution.

Recording of DR 5-HT neurons. Single glass micropipettes pulled on a pipette puller (Narishige, Tokyo, Japan) with impedances ranging from 2.5 to 5 m Ω , were positioned 0.2 to 0.5 mm posterior to the interaural line on the midline and lowered into the DR, usually attained at a depth between 2.5 and 3.5 mm from the brain surface (Franklin and Paxinos, 2007). The DR 5-HT neurons were identified using the following criteria: a slow (0.5–2.5 Hz) and regular firing rate and a long duration, positive action potential (Aghajanian and Vandermaelen, 1982). The total number of spontaneously active 5-HT neurons and their firing rates were determined by monitoring their average discharge frequency. In each mouse, several tracts were performed to measure the spontaneous firing rate of DR 5-HT neurons. At the end of the experiment, only one neuron was studied with 8-OHDPAT (100–300 μ g/kg; s.c.) to assess the functional activity of somatodendritic 5-HT_{1A} autoreceptor. WAY100635 was used to reverse the suppressant effect of 8-OHDPAT on the firing activity of DR 5-HT neurons. Changes in the firing activity were expressed as percentage of baseline firing rate.

In vivo microdialysis.

Concentric dialysis probes (active length of 1 mm) were made of cuprophan fibres and set up as described previously (Malagié et al., 1996). Animals were anaesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic frame for probe implantation in the dorsal raphe (DR) according to the ‘Mouse brains’ atlas (Franklin and Paxinos, 2007): coordinates from Bregma (in mm): anterior, – 4.5; lateral, 0; ventral; 3.0. Animals were allowed to recover from surgery overnight. The next day, ~20 h after the surgery, probes were continuously perfused with artificial cerebrospinal fluid (composition in mmol/L: NaCl 147, KCl 3.5, CaCl₂ 1.26, MgCl₂ 1.2, NaH₂PO₄ 1.0, pH 7.4 \pm 0.2) at a flow rate of 1 μ l/min using CMA/100 pump (Carnegie Medicin, Stockholm, Sweden). Dialysates were collected every 30 min in small Eppendorf tubes for the measurements of their 5-HT contents ([5-HT]_{ext}) using a

high performance liquid chromatography (HPLC) system. Four fractions were collected to measure basal values (means \pm SEM) and 4 subsequent fractions were collected after administration of 8-OHDPAT (100 μ g/kg; s.c.). The limit of sensitivity for [5-HT]_{ext} was 0.5 fmol per sample (signal-to-noise ratio : 2). At the end of the experiments, the placement of microdialysis probes was verified histologically.

Data analysis and statistics.

Results from data analyses were expressed as mean \pm SEM of body temperature (behavior), of DR 5-HT firing rate (electrophysiology) and of [5-HT]_{ext} in the DR (neurochemistry). Two-way ANOVAs were applied for the statistical analyses of the data with pre-treatment (vehicle *vs* corticosterone) and treatment (vehicle *vs* fluoxetine) as main factors. In all cases, when appropriate, pairwise comparisons were performed using the Protected at Least Significance (PLSD) post-hoc test using the computer software Stat-View 5.0. Accepted level of significance was set at $p \leq 0.05$.

Results and discussion

Because 5-HT_{1A} autoreceptor is a key component in the regulation of serotonergic neurotransmission, the present study examined its functional status after chronic administration of fluoxetine in mice pre-treated with corticosterone or vehicle.

In mice, 8-OHDPAT-induced hypothermia is mediated by 5-HT_{1A} autoreceptor (Richardson-Jones et al., 2010). We used this response to assess the functional status of this receptor in our experimental groups. A two-way ANOVA on body temperature revealed a significant effect of pre-treatment [$F(1,36)=15.0$, $p < 0.001$] and treatment factors [$F(1,36)=57.8$, $p < 0.001$]. 8-OHDPAT significantly decreased the body temperature of vehicle- and corticosterone-pre-

treated mice (Figure 1) but this response was less pronounced in the latter group suggesting a functional desensitization of 5-HT_{1A} autoreceptor. These findings are consistent with initial studies showing that maternal separation or UCMS in rodents (Bambico et al., 2009; Froger et al., 2004; Gartside et al., 2003; Lanfumey et al., 1999), reduced 5-HT_{1A} autoreceptor sensitivity. When fluoxetine was given for 28 days, the hypothermic response to 8-OHDPAT was also significantly attenuated in both vehicle- and corticosterone-pre-treated mice (Figure 1), but this blunted response was greater in corticosterone-pre-treated mice. Interestingly, a hyperthermic response was even detected in the latter group of mice suggesting that 8-OHDPAT also mobilized a population of 5-HT_{1A} receptors that differs from that involved in hypothermia (Olivier et al., 2008).

To confirm the possibility that the effects of fluoxetine on 5-HT_{1A} autoreceptor sensitivity are potentiated in corticosterone-pre-treated mice, we examined the potency of 8-OHDPAT to inhibit the firing activity of DR 5-HT neurons. A two-way ANOVA on the percentage of inhibition of basal 5-HT firing rates induced by 8-OHDPAT, revealed a significant effect of pre-treatment [$F(1,28)=13.3$, $p<0.01$] and treatment factors [$F(1,28)=34.2$; $p<0.001$]. As expected from the above-mentioned on body temperature, 8-OHDPAT was less potent in suppressing 5-HT neuronal firing activity in corticosterone- compared to vehicle-pre-treated mice (ED_{50} were 205 and 110 $\mu\text{g}/\text{kg}$, respectively; Figure 2A). When fluoxetine was given for 28 days, the suppression of DR 5-HT neuronal activity induced by 8-OHDPAT was significantly lower in corticosterone- compared to vehicle-pre-treated mice (Figures 2A-2C). Having established that the potency of fluoxetine to desensitize 5-HT_{1A} autoreceptor is enhanced in corticosterone-pre-treated mice, we then determined whether this adaptive change influenced the basal firing rate and the number of spontaneously active 5-HT neurons in the DR. A two-way ANOVA on the basal firing rate of DR 5-HT neurons revealed a significant effect of pre-treatment [$F(1,186)=3.6$, $p<0.05$], treatment factors [$F(1,186)=28.5$,

$p < 0.001$] and an interaction between both variables [$F(1,186) = 8.7$, $p < 0.001$]. Figures 2C and 2D show that the mean firing activity of DR 5-HT neurons is similar between vehicle- and corticosterone-pre-treated mice (1.8 ± 0.1 Hz and 2.1 ± 0.1 Hz; $p > 0.05$, respectively). This stands in contrast with recent data reporting that the mean spontaneous single-spike firing rate of 5-HT neurons in rats submitted to UCMS was lower than that of the control group (Bambico et al., 2009). Nevertheless, our results concur with data reporting that the basal firing rate of DR 5-HT neurons in rats is not affected by chronic corticosterone treatment despite the desensitization of 5-HT_{1A} autoreceptor (Fairchild et al., 2003). It thus appears that the degree of stress may play a major role in the regulation of DR 5-HT neuronal activity. Consistent with this hypothesis, Bambico et al. (2009) reported that chronic unpredictable stress reduced the firing rate of DR 5-HT neurons while acute restraint stress failed to do so. In the present study, the observation that the basal DR 5-HT neurons firing rate was not altered in corticosterone mice despite the desensitization of 5-HT_{1A} autoreceptor may be attributable to the lack of tonic activation of this receptor *in vivo* (Bortolozzi et al., 2004; Guilloux et al., 2006). It is also possible that compensatory mechanisms such as a hypersensitization of the terminal 5-HT_{1B} autoreceptor occurred (Gur et al., 2001). After 28 days of treatment with fluoxetine, a significant decrease in the firing activity of 5-HT neurons was observed in vehicle- (1.1 ± 0.1 Hz), but was no longer present in corticosterone-pre-treated mice (2.3 ± 0.2 Hz) when compared to corresponding groups of mice administered with vehicle (Figures 2C-D). Thus, the recovery in firing rate of DR 5-HT neurons returned here to baseline after 28 days of fluoxetine treatment specifically in corticosterone-pre-treated mice. Hence, one of the most remarkable results obtained herein is the observation that the combination of both agents produced additional effects allowing 5-HT neurons to regain their baseline more rapidly when it has been first sensitized (or activated) by the elevated corticosterone levels. This result is in agreement with a robust desensitization of 5-HT_{1A}

autoreceptor and suggests that pathological conditions are necessary for fluoxetine to produce its maximal electrophysiological effects. With respect to the number of spontaneous active DR 5-HT neurons, a two-way ANOVA revealed no significant effect of pre-treatment [$F(1,13)=1.1$, $p>0.05$] and treatment [$F(1,15)=0.7$, $p>0.05$] factors. The number of neurons recorded per electrode descent is known as a valid, indirect index of the percentage of neurons that are spontaneously discharging (active) during *in vivo* electrophysiological recordings. Nevertheless, in the present study the number of spontaneously active DR 5-HT neurons was not modified either by corticosterone and/or fluoxetine treatments (Table 1).

Having observed electrophysiological differences between corticosterone- and vehicle-pre-treated mice administered with fluoxetine on the sensitivity of 5-HT_{1A} autoreceptor, we next asked how these differences are reflected at the neurochemical level by using *in vivo* microdialysis at somatodendritic level (i.e., in the DR). A two-way ANOVA on the percentage of inhibition of 5-HT extracellular levels induced by 8-OHDPAT, revealed a significant effect of pre-treatment [$F(1,16)=12.1$, $p<0.01$] and treatment factors [$F(1,16)=5.1$, $p<0.05$]. Our results show that the decrease in the extracellular levels of 5-HT in the DR induced by 8-OHDPAT was significantly lower in corticosterone- than in vehicle-pre-treated mice administered with fluoxetine (Figures 3A-B). Once again, our results emphasize the fact that the functional desensitization of 5-HT_{1A} autoreceptor in response to fluoxetine is potentiated in corticosterone-pre-treated mice. It is noteworthy that AUC values on extracellular levels of 5-HT in the DR parallel hypothermia data suggesting that such response may be an indirect measure of changes in basal 5-HT outflow in this brain region. Regarding basal extracellular levels of 5-HT in the DR, a two-way ANOVA revealed no significant effect of pre-treatment [$F(1,16)=1.7$, $p>0.05$], but a significant effect of treatment factors [$F(1,16)=28.6$, $p<0.001$]. The absence of differences on the basal extracellular levels of 5-HT in the DR between vehicle- and corticosterone-pre-treated mice is consistent with

electrophysiological data. As expected, fluoxetine significantly increased basal extracellular levels of 5-HT in the DR in both vehicle and corticosterone-pre-treated mice (Table 2), with a trend of higher increase in the corticosterone group of mice. These findings raise the possibility that the degree of increase in 5-HT levels in the vicinity of 5-HT cell bodies might account for the differential degree of desensitization of 5-HT_{1A} autoreceptor. This neurochemical observation concurs with a higher firing rate of DR 5-HT neurons and a more pronounced desensitization of 5-HT_{1A} autoreceptor in corticosterone- compared to vehicle-pre-treated mice in response to fluoxetine. However, previous microdialysis data failed to detect differences in basal cortical extracellular levels of 5-HT between sham and corticosterone-treated rats after chronic fluoxetine administration (Gartside et al., 2003). It is thus possible that the nature of the neurochemical effects of corticosterone on extracellular levels of 5-HT may depend on the brain region studied (somatodendritic vs terminal areas), the mode of corticosterone administration (drinking water vs pellet) and/or the species studied (rats vs mice).

The present study performed in mice demonstrates that chronic administration of corticosterone in the drinking water i) produces a functional desensitization of 5-HT_{1A} autoreceptor without affecting the basal firing rate or extracellular levels of 5-HT in the DR, ii) potentiates fluoxetine-induced desensitization of 5-HT_{1A} autoreceptor. Such mechanism is in favor of a greater enhancement of brain serotonergic neurotransmission and would thus represent a plausible explanation of the antidepressant-like effects of SSRI, specifically in animal models of anxiety/depression. Several hypotheses may explain the fact that corticosterone desensitized 5-HT_{1A} autoreceptor and potentiated the behavioral, electrophysiological and neurochemical effects of fluoxetine. It is possible that corticosterone directly downregulated the expression of 5-HT_{1A} autoreceptor. *In situ* hybridization and immunocytochemical studies have revealed the presence of Glucocorticoid Receptor (GR)

mRNA or protein specifically within 5-HT cell bodies in the DR (Härfstrand et al., 1986) while the 5-HT_{1A} receptor gene includes a glucocorticoid responsive element (GRE) (Ou et al., 2001). Therefore, corticosterone might have desensitized somatodendritic autoreceptor through a mechanism independently to an increase in extracellular 5-HT levels in the DR. Interestingly, it was shown that corticosterone significantly reduced the expression of mRNA encoding G-protein linked inwardly rectifying K⁺ (GIRK) channel (Fairchild et al., 2003) suggesting that the desensitization of 5-HT_{1A} autoreceptor induced by corticosterone could result from an alteration in their coupling property. Another possibility would be that corticosterone increased the activity of the tryptophan hydroxylase. The activation of TpH would thus result in an increase in 5-HT release at somatodendritic levels, thereby facilitating the functional inactivation of 5-HT_{1A} autoreceptor. Nevertheless, in the present study although corticosterone enhanced the effect of fluoxetine on basal extracellular levels of 5-HT, it had no effect on this parameter when given alone. Finally we cannot rule out the possibility that corticosterone, as observed with SSRIs, decreased 5-HT reuptake. In line with this hypothesis, a recent study has shown that corticosterone blocks the reuptake of 5-HT through low-affinity monoamine transporters (Baganz et al., 2010). This point should draw our attention for future investigations.

Authorship contributions

Participated in the design of the study: Quentin Rainer, Hai T. Nguyen, Gaël Quesseveur, Alain M. Gardier, Denis J. David, Bruno P. Guiard B

Conducted experiments: Quentin Rainer, Hai T. Nguyen, Gaël Quesseveur, Bruno P. Guiard

Contributed new reagents or analytic tools: Gaël Quesseveur

Performed data analysis: Quentin Rainer, Hai T. Nguyen, Bruno P. Guiard B

Wrote or contributed to the writing of the manuscript: Quentin Rainer, Hai T. Nguyen, Gaël Quesseveur, Alain M. Gardier, Denis J. David, Bruno P. Guiard B

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Footnotes

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Legends for figures

FIGURE 1: Effect of sustained administration of corticosterone ± fluoxetine on 8-OHDPAT-induced hypothermia. Data are means ± SEM of decrease in body temperature (°C) measured 10 min after 8-OHDPAT (100 µg/kg; s.c.) administration. *p<0.05 and ***p<0.001: significantly different from vehicle (VEH) / vehicle (VEH) treated mice. \$\$\$p<0.001: significantly different from vehicle (VEH) / corticosterone (CORT) treated mice. ##p<0.01: significantly different from vehicle (VEH) / fluoxetine (FLX) treated mice (n=10 mice per group).

FIGURE 2: Effect of sustained administration of corticosterone ± fluoxetine on dorsal raphe 5-HT neuronal activity. All dorsal raphe (DR) 5-HT neurons tested were inhibited, in a dose-dependent manner by 8-OHDPAT (100-300 µg/kg, s.c.), this inhibition being reversed by the administration of the selective 5-HT_{1A} receptor antagonist WAY100635 (300 µg/kg; s.c.). **(A)** Data are means ± SEM of inhibition in percentage of baseline. These means were measured on the 60-s period preceding each 8-OHDPAT administration in vehicle (VEH)- (○, ●) or corticosterone (CORT)- (□, ■) pre-treated mice administered with vehicle (VEH: white symbols) or fluoxetine (FLX, 18 mg/kg/28days; po: black symbols). **(B)** Data are expressed as area under the curve (AUC; mean ± SEM). AUC values were calculated for the inhibitory effect of 8-OHDPAT on DR 5-HT neuronal activity and expressed as percentage of baseline. **p<0.01 and ***p<0.001: significantly different from vehicle (VEH) / vehicle (VEH) treated mice. \$\$p<0.01: significantly different from vehicle (VEH) / corticosterone (CORT) treated mice. #p<0.05: significantly different from vehicle (VEH) / fluoxetine (FLX) treated mice (n=4-6 mice per group). (n=5-9 mice per group). **(C)** Examples of typical recordings of DR 5-HT neurons obtained in each experimental group. **(D)** Data are means ±

SEM firing rate in Hertz (Hz) (n=3–7 mice per treatment group). The numbers within the histograms indicate the number of neurons recorded. ***p<0.001, significantly different from the firing rate of DR 5-HT neurons in vehicle (VEH) / vehicle (VEH) treated mice. ###p<0.001, significantly different from vehicle (VEH) / fluoxetine (FLX) treated mice. **(E)** Distribution of 5-HT single spike firing activity per treatment group.

FIGURE 3: Effect of sustained corticosterone ± fluoxetine on extracellular levels of 5-HT in the dorsal raphe. **(A):** Effect of systemic administration of 8-OH-DPAT on extracellular levels of 5-HT ([5-HT]_{ext}) in the dorsal raphe (DR) in vehicle (VEH)-(○, ●) or corticosterone (CORT)-(□, ■) pre-treated mice administered with vehicle (VEH: white symbols) or fluoxetine (FLX, 18 mg/kg/28days; po: black symbols). Results are expressed as means ± SEM of [5-HT]_{ext} (percentages of basal values). Mice received (arrow) 8-OH-DPAT (100 µg/kg; s.c.). **(B)** Data are expressed as area under the curve (AUC; mean ± SEM). AUC values were calculated for the amount of 5-HT outflow measured in the DR during the 0–120 min post-treatment period with the 5-HT_{1A} receptor agonist 8-OH-DPAT and expressed as percentages of baseline. *p<0.05: significantly different from vehicle (VEH) /vehicle (VEH) treated mice. ##p<0.01: significantly different from vehicle (VEH) / fluoxetine (FLX) treated mice (n=4-6 mice per group).

TABLE 1

| Pretreatment | Treatment | Number of neuron/tract |
|----------------------------------|-------------------------|-----------------------------------|
| Vehicle (β -CD 0.45%) | Vehicle | 3.1 \pm 0.6 |
| Vehicle (β -CD 0.45%) | Fluoxetine (18 mg/kg/d) | 2.7 \pm 0.2 |
| Corticosterone (35 μ g/ml/d) | Vehicle | 3.5 \pm 0.3 |
| Corticosterone (35 μ g/ml/d) | Fluoxetine (18 mg/kg/d) | 2.9 \pm 0.3 |

Effect of sustained corticosterone \pm fluoxetine on the number of spontaneously active DR 5-HT neurons.

TABLE 2

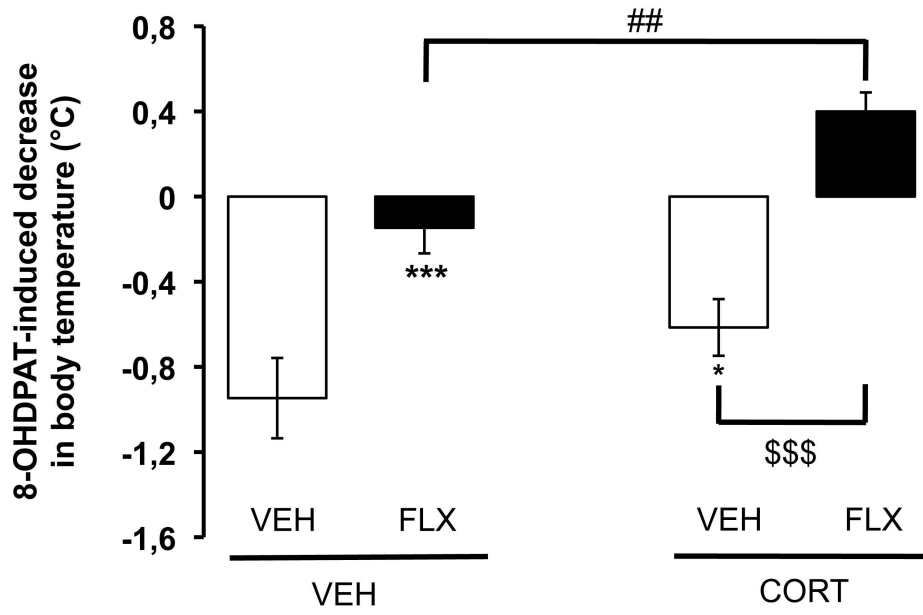
| Pretreatment | Treatment | Basal extracellular 5-HT in the DR (fmol) |
|----------------------------------|-------------------------|--|
| Vehicle (β -CD 0.45%) | Vehicle | 13.4 \pm 4.1 |
| Vehicle (β -CD 0.45%) | Fluoxetine (18 mg/kg/d) | 75.9 \pm 3.1*** |
| Corticosterone (35 μ g/ml/d) | Vehicle | 14.8 \pm 3.6 |
| Corticosterone (35 μ g/ml/d) | Fluoxetine (18 mg/kg/d) | 114.9 \pm 24.1** |

Basal extracellular 5-HT levels in the DR in vehicle/vehicle; vehicle/fluoxetine, corticosterone/vehicle and corticosterone/fluoxetine groups of mice. Data are the mean \pm SEM calculated from 4 baseline samples. **p<0.01 and ***p<0.001: significantly different from the corresponding group of mice treated with vehicle (VEH). (n=4-6 mice per group).

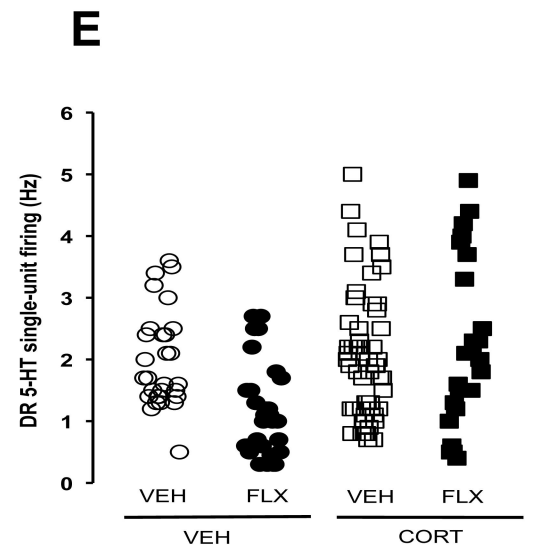
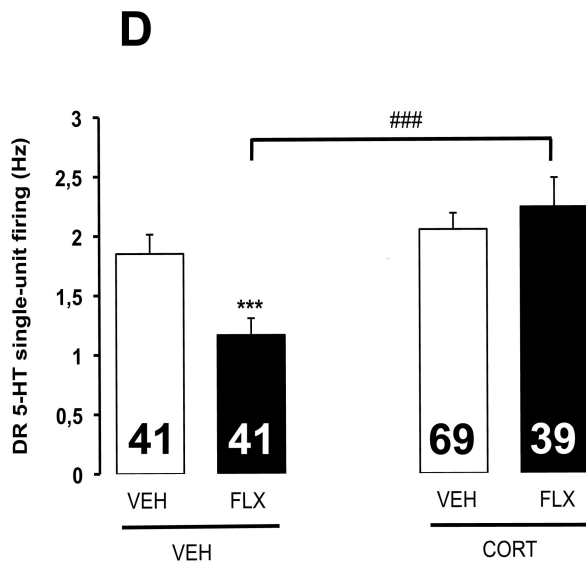
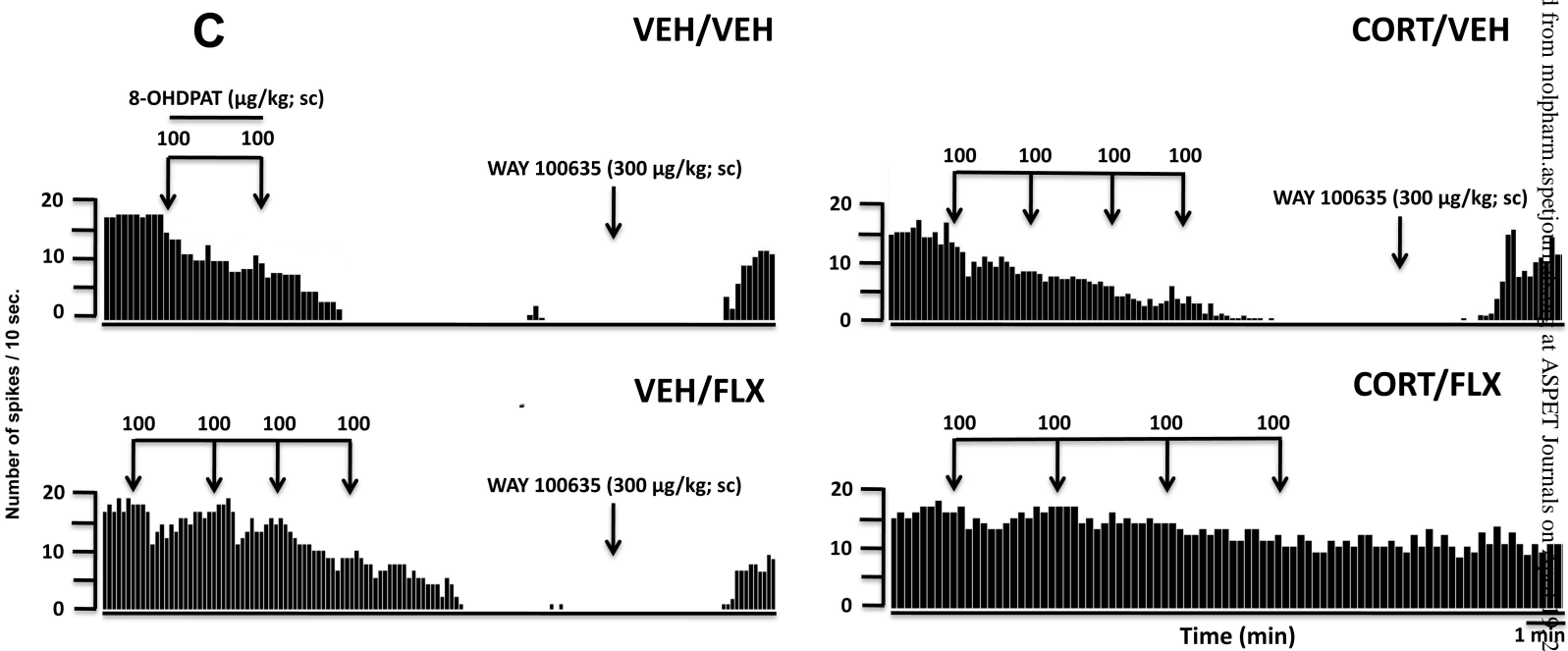
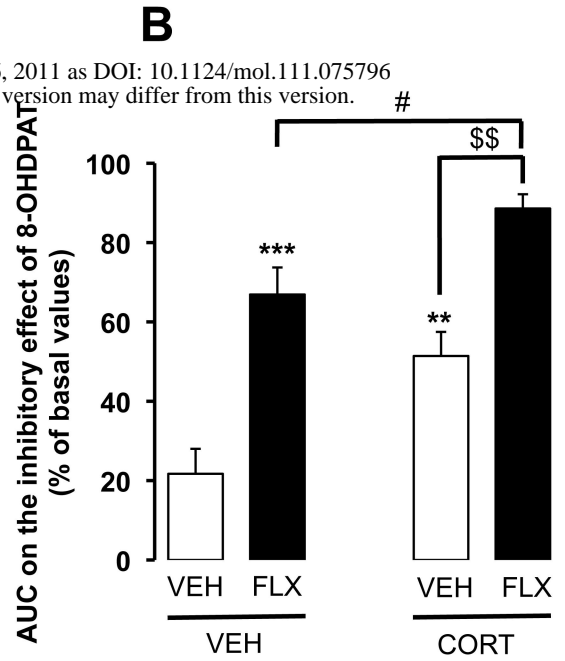
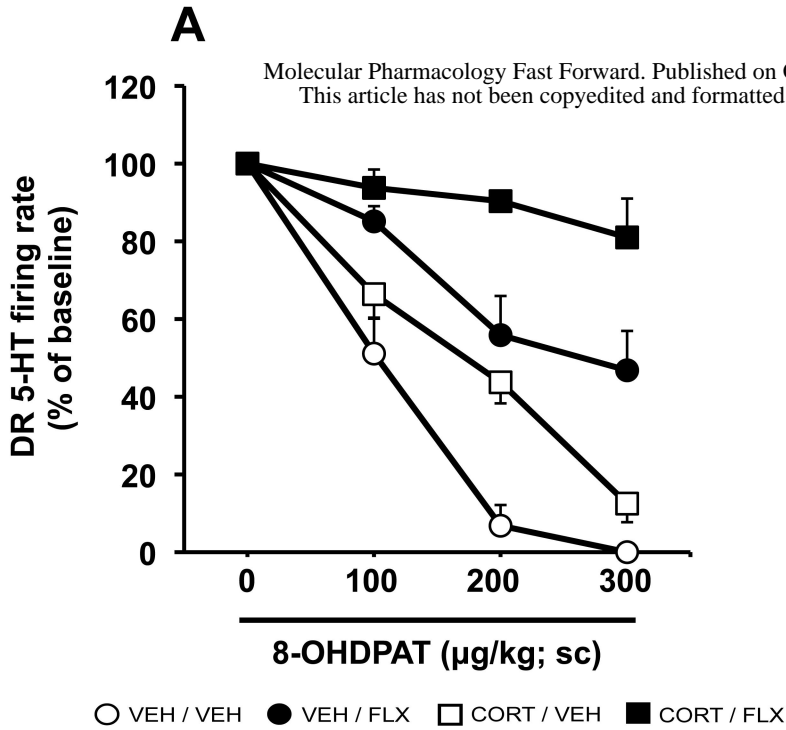
FIGURE 1

HYPOTHERMIA

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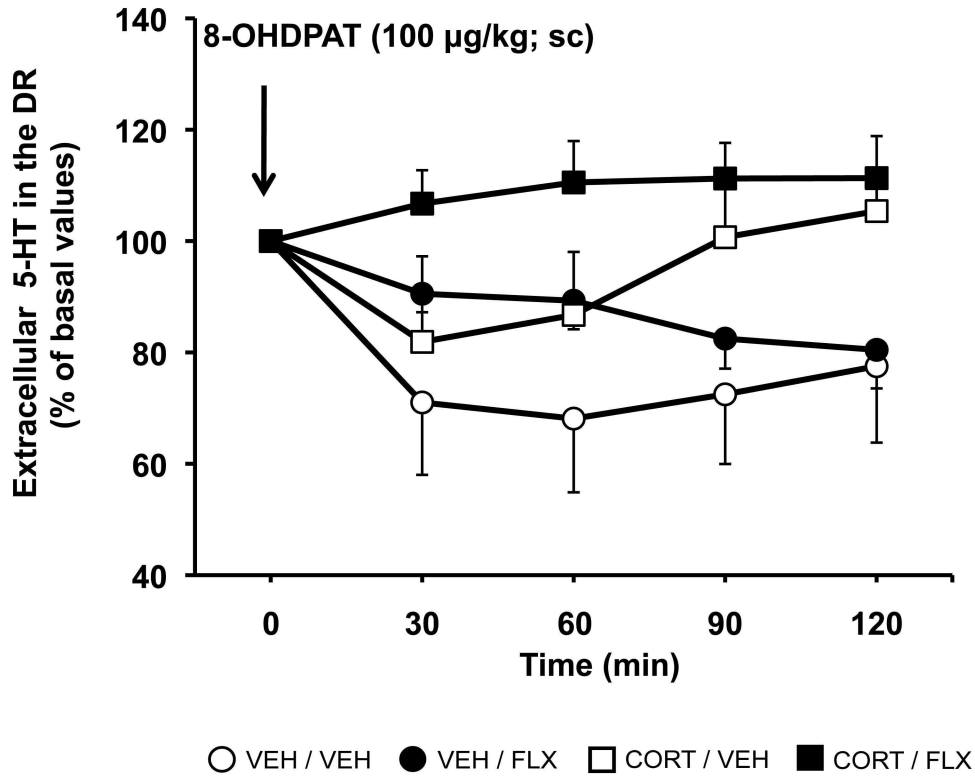


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