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Investigating the Neurobiology of Motivational Deficiencies in Major Depressive Disorder: 5-HT1B Receptor Involvement in Behavioral Effects of Fluoxetine (Prozac)

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Investigating the neurobiology of motivational deficiencies in Major Depressive Disorder:

5-HT1B receptor involvement in behavioral effects of fluoxetine (Prozac)

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Abstract

Selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine (Prozac) are the most commonly prescribed treatments for depression. Although efficacious for symptoms such as a depressed mood, SSRIs do not alleviate symptoms of amotivation, anergia, and fatigue. Furthermore, in clinical and preclinical studies, SSRIs have been shown to exacerbate motivational impairments and general fatigue. It is likely that fluoxetine-induced dysfunctions are due to overstimulation of one or more 5-HT receptors, with one possible candidate being the 5-HT$_{1B}$ receptor. Therefore, the aim of the study was to evaluate the role of 5-HT$_{1B}$Rs in fluoxetine-induced amotivation using a rodent behavioral model of effort-based decision-making. For these experiments, the selective 5-HT$_{1B}$R antagonist, NAS-181, was co-administered with fluoxetine to determine if fluoxetine-induced suppression of high effort behavior could be attenuated. NAS-181 partially reversed the effects of fluoxetine in rats that showed a greater fluoxetine-induced behavioral suppression in our task, which was not found in rats with low fluoxetine-induced suppression. Future directions involve intracranial administration and investigation of the role of other 5-HTRs on effort-related behaviors.

Keywords: depression, 5-HT$_{1B}$R, motivational impairment, SSRI

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Major depressive disorder (MDD) is a common psychiatric disorder, with roughly 7.6% of Americans over the age of 12 suffering from MDD in a given 2-week period[15]. Not only is MDD highly prevalent, but it is also incredibly debilitating, with the World Health Organization ranking it as the leading cause of disease burden across both low and high income countries[22]. Motivational symptoms in particular (e.g. psychomotor retardation, lack of energy, low exertion of effort, fatigue) severely impair important aspects of a patient's life and tend to be the most treatment resistant aspects of MDD[18]. Because of its prevalence and severity as a worldwide health concern, the need for effective treatments is extremely great.

For these reasons, considerable work has focused on developing a greater understanding of the neurobiology of the motivational aspects of MDD, with a particular focus on preclinical studies of potential treatments that affect effort-related decision-making. Over the past few decades, behaviorally validated rodent models of effort-related motivational impairments have been developed. These assays quantify effort exertion in animals by offering a choice between obtaining a highly preferred reward through high exertion of effort behaviors (e.g. lever pressing or barrier climbing) vs. low-effort options leading to less preferred rewards[18]. In rodents, conditions linked to depression (e.g. stress, inflammatory, or pharmacological challenge) can induce a low-effort bias, which is consistent with the results of human studies on effort-based choice in MDD patients[7,18]. For example, the vesicular monoamine transport type 2 (VMAT-2) inhibitor tetrabenazine (TBZ), which causes depressive symptoms in people, can produce a low-effort decision bias in rats that can be reversed by drugs that can improve motivational symptoms in people, such as bupropion and methylphenidate[13]. Bupropion and methylphenidate inhibit the

reuptake of catecholamines (dopamine (DA) and norepinephrine), which causes an increase in the extracellular concentrations of these neurotransmitters[13]. These DAergic mechanisms appear to be crucial in determining the ability of a drug to improve effort-related processes, which is consistent with recent clinical studies focusing on the importance of nucleus accumbens DA for regulating motivational function[18,23,25]. However, DA does not regulate these effort-related functions alone, and there is evidence of interactions between DA and several other neurotransmitters, including GABA, serotonin (5-HT), and adenosine.

To illustrate how other neurotransmitters can exhibit influences on DAergic actions, some of our previous research in the Salamone lab has focused upon interactions between DA and adenosine[17]. Adenosine A_{2A} receptors are co-localized with DA D2 receptors in DA-rich striatal areas of the brain (such as neostriatum and nucleus accumbens) and have been found to influence the post-synaptic effects of DA[14,19]. Moreover, many studies have demonstrated that by inhibiting adenosine A_{2A} receptors, the motivational effects of DA antagonists and depletions can be reversed[5,14,21]. Preladenant (PLD), an adenosine A_{2A} receptor antagonist, was initially developed as a possible treatment for Parkinson's Disease and is much more selective (about 1000-fold more selective) for the A_{2a} receptor compared to other adenosine receptors[1,8]. Therefore, to evaluate the potential of PLD for the treatment of MDD in a previous study, we used a concurrent operant choice procedure and were able to demonstrate that PLD can reverse the effort-related impairment induced by TBZ[17].

Current research in the Salamone lab also is focused on the role of 5-HT, and interactions between DA and 5-HT, in animal models of depression and other psychiatric disorders. The most commonly prescribed antidepressants are selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine (Prozac)[18]. While these drugs can treat a variety of depressive symptoms (e.g. mood,

rumination, and anxiety), effort-related motivational symptoms tend to be much more resistant to treatment with SSRIs[6,18]. In rat studies of effort-related decision making, the SSRIs fluoxetine and citalopram failed to reverse the effort-related effects of TBZ[6,23,25]. Not only has fluoxetine been found to be relatively ineffective in reversing motivational deficiencies in both clinical studies and preclinical rodent models, but it has even been found to exacerbate motivational deficits in rats and humans when administered alone[6,18,23,24,25]. Fluoxetine was recently shown to decrease selection of high-effort lever pressing activity on a progressive ratio/chow feeding choice task (PROG), a procedure in which the lever pressing work requirement progressively increases causing rats to eventually switch from high-effort lever pressing to the low effort alternative[24]. In addition to reducing selection of high-effort behavior in rats, fluoxetine also decreased extracellular DA in the nucleus accumbens[24]. As the mechanism of action of SSRIs involves elevating extracellular levels of serotonin (5-HT) by blocking 5-HT reuptake, it is likely that the motivational dysfunctions induced by fluoxetine are due to an overstimulation of one or more 5-HT receptors. Supporting this, a recent study determined that enhancing 5-HT transmission from the dorsal raphe nucleus (DRN) to the VTA results in a decrease in striatal DA[2].

  There are several subtypes of 5-HT receptors in the brain (i.e., 5-HT1-7, with additional subtypes within some of these families of receptors). However, despite the direct localization of 5-HT2 family receptors on DA neurons in the mesolimbic pathway, previous findings have obtained signify that the behavioral functions of $5\text{-}HT_{2A}$ and $5\text{-}HT_{2C}$ receptors do not appear to be involved in fluoxetine induced amotivation (Rotolo et al., in preparation). Therefore, it is critical to shift our focus to a broader range of 5-HT receptors in order to individually characterize their functions and possible contributions to effort-related behavior.

The 5-HT$_{1B}$ receptor has been implicated in various behavioral effects in rodent models. Nautiyal et al. have shown that 5-HT$_{1B}$ receptors in the ventral tegmental area (VTA) appear to play a role in the modulation of impulsive behaviors, and evidence indicates that this is possibly due to actions on the mesolimbic DA neurons that originate in VTA and project to the nucleus accumbens[11]. Therefore, it is possible that 5-HT$_{1B}$ receptors may be able to mediate aspects of motivation via the same interactions. Consistent with this idea, 5-HT$_{1B}$ autoreceptors are localized in the DRN, which has projections to the VTA and has been implicated in the regulation of striatal DA[2,9]. Furthermore, knockout mice for 5-HT$_{1B}$ autoreceptors have demonstrated a reduction in depressive and anxiety-like behavior[12]. For these reasons, there is particular interest in the 5-HT$_{1B}$ receptor when considering aspects of motivated behavior. Therefore, the aim of this study was to determine whether a 5-HT$_{1B}$R antagonist, NAS-181 (NAS), could reverse the low effort bias induced by fluoxetine in a rodent model which would implicate a role for 5-HT$_{1B}$ receptors in the mechanisms of fluoxetine.

Methods

Animals.

11 male Sprague Dawley rats with no prior drug experience were obtained from Harlan Laboratories, Indianapolis, IN, USA, initially weighing about 279-299 g. Rats were pair housed in a colony maintained at 23°C with a 12 hr light/dark cycle (lights on 07:00). Rats were food restricted to 85% of their free-feeding body weight for initial training, and allowed modest growth during the study. Water access in the home cages was unrestricted. All animal procedures have been approved by the University of Connecticut Institutional Animal Care and Use Committee (Salamone Lab Protocol: A17017).

Behavioral Task.

The experiment used a fixed ratio of 5 (FR5)/chow feeding choice task to assess effort-related decision making. With this task, rats are given the choice between working for preferred high carbohydrate food pellets (45 mg; Bio-Serv, Frenchtown, NJ) by lever pressing on a FR5 schedule (5 presses delivers one pellet) vs. approach and consumption of the less preferred standard lab chow. Behavioral testing sessions (30 min session, 5 days/week) were conducted in Med Associates operant chambers during the light part of the light/dark cycle at the same time each day. Rats were trained initially to lever press for pellets on a FR1 schedule for one week, after which they were switched to an FR5 schedule for roughly four weeks. After reaching stable baseline lever presses per session, concurrently available laboratory chow was introduced to the operant chambers for the FR5/chow feeding choice task.

Drugs.

Subcutaneous injections of the selective $5\text{-}HT_{1B}$ antagonist NAS-181 (Tocris Bioscience), co-administered with fluoxetine (FLX), was used to determine if the $5\text{-}HT_{1B}$ receptor is responsible for the reduced selection of high-effort FR5 lever pressing induced by FLX. FLX was dissolved in 0.9% saline which was also used as the vehicle control, and was administered at 12.5 mg/kg 90 minutes before testing. NAS-181 (1.0-8.0 mg/kg) or its 0.9% saline vehicle control was administered 50 minutes before testing. A repeated measures design was used, meaning each animal received all combinations of drug treatments once per week in a randomly varied order. The drug treatments were: FLX vehicle + NAS-181 vehicle (VEH/VEH), 12.5 mg/kg FLX + NAS-181 vehicle (FLX/VEH), 12.5 mg/kg FLX + 1.0 mg/kg NAS-181 (FLX/NAS 1), 12.5 mg/kg FLX + 2.0 mg/kg NAS-181 (FLX/NAS 2), 12.5 mg/kg FLX + 4.0

mg/kg NAS-181 (FLX/NAS 4), and 12.5 mg/kg FLX + 8.0 mg/kg NAS-181 (FLX/NAS 8) (6 total treatments).

*Data Analysi*s.

Repeated measures analysis of variance (ANOVA) was calculated using SPSS. For some analyses, and rats were split into groups based on the degree of suppression induced by FLX. To determine sources of significant effects we used non-orthogonal planned comparisons using the overall error term from each analysis. Power analysis was also used to determine the group size.

Results

To assess the possible role of 5-HT_{1B} receptors on motivational impairments associated with FLX, we investigated the behavioral effects of NAS coadministration with FLX on lever pressing and chow consumption in a FR5/chow concurrent choice task. Analysis of chow intake revealed no significant differences between any of the treatments (data not shown). Initial analysis of lever pressing demonstrated a significant reduction in behavior upon FLX/VEH administration, however coadministration of FLX with various NAS doses appeared to produce no significant differences compared to FLX/VEH (data not shown). However, we did observe great variation regarding the magnitude of FLX suppression between individual subjects.

Therefore, we used a criterion of 75% suppression of lever pressing from baseline to separate those experiencing a large amount of FLX behavioral suppression from those that only experience moderate to low changes in behavior. The resulting "high FLX suppression" (n=6) and "low FLX suppression" (n=5) cohorts each had less variation of lever pressing suppression than when the two different groups were treated as a whole. To illustrate how fundamentally different these two cohorts were regarding their behavioral suppression, the high FLX

suppression cohort averaged a FLX-induced suppression in pressing from vehicle that was upwards of 90% whereas that of the low suppression cohort fell short of 45%.

Proceeding with our planned analyses, instead treating the two groups as the distinct groups they are, revealed two contradicting behavioral profiles (Figure 1). While both cohorts displayed significance in FLX/VEH suppression from VEH/VEH baseline lever pressing $[F_{(1,25)}=33.255 \ p<0.01; F_{(1, 20)}=7.125 \ p<0.025]$, NAS administration with the high suppression cohort produced a partial but significant reversal of the pharmacological challenge induced by FLX $[F_{(1,25)}=4.580, p<0.05]$ (Figure 1A). NAS administration for the low suppression cohort, however, resulted in a significant attenuation of lever pressing $[F_{(1,20)}=5.328, p<0.05]$ (Figure 1B). Additionally, the quadratic trend is significant when assessing the interaction between the two groups $[F_{(1,9)}=6.921 \ (p<0.05, p=0.027)]$. The chow intake once again displayed no significant differences across treatment groups in either the high suppression or low suppression cohorts (data not shown).

Discussion

The goal of the study was to determine if $5\text{-HT}_{1B}R$ are implicated in the mechanisms of FLX-induced amotivation. Through $5\text{-HT}_{1B}R$ antagonism, we were able to obtain a partial restoration of lever pressing behavior from the effects of FLX in a subset of rats, which signifies that $5\text{-HT}_{1B}R$ do appear to play at least a partial role in the impairments of FLX. As individual variance in the magnitude of FLX-induced amotivation actually appears to be predictive of the behavioral effects of serotonin antagonism, we also looked at previous results we had obtained from similar studies investigating 5-HT_2 family antagonists. However, utilizing the same method of cohort separation as well as the same criterion, we were unable to observe the same

phenomenon. This supports the idea that the effect we identified in this experiment truly is unique to the 5-HT$_{1B}$R.

Although we obtained no compensatory increase in chow intake due to decreased lever pressing, this is consistent with our previous studies using FLX and is thought to occur due to the appetite suppressant effects of the drug. Furthermore, the behavioral change due to FLX administration does appear to be motivational in nature rather than appetitive as experiments our lab has conducted using nonfood reinforces also see a shift to a low effort preference as well.

It is possible that the behavioral differences observed between these two cohorts may be indicative of differences in receptor tone. Therefore, the proposed biological basis underlying FLX-induced amotivation seen in patients may also involve differential expression patterns of the 5-HT$_{1B}$R. Further studies are required to validate this prediction however. Namely, histological analysis of 5-HT$_{1B}$R expression throughout the DRN or possibly neurons in the VTA.

To further understand whether 5-HT$_{1B}$Rs specifically in the DRN and VTA are involved in FLX-induced motivational suppression, it would be important to conduct further investigations utilizing intracranial NAS-181 administration directly to these areas. This could potentially reveal a greater behavioral effect than those seen with systemic administration as there would be less recruitment of extraneous serotonergic systems. Alternatively, if no effect is seen through the involvement of these areas alone, alternative areas such as the frontal cortex may be more greatly involved than we initially thought.

We are currently investigating whether NAS-181 administration alone may be increasing high effort behavior using a progressive ratio choice study and also plan to evaluate whether 5-HT$_{1B}$R antagonism may be resulting in changes of extracellular DA concentrations in the NAc. These studies will involve using microdialysis methods and high performance liquid

chromatography with electrochemical detection with the ultimate goal to understand if $5\text{-HT}_{1B}R$

mediates the behavioral outcomes we observed through DAergic actions. If not, $5\text{-HT}_{1B}R$ also

are known to serve as heteroreceptors regulating transmission of acetylcholine, glutamate,

norepinephrine and GABA, any of which may also be involved in this behavioral outcome[9]. In

this vein, we also have underway a study coadministering FLX with PCPA, a tryptophan

hydroxylase inhibitor which would downregulate 5-HT synthesis overall. This would provide

some insight as to whether 5-HT truly is the primary mediator of FLX-induced amotivation, and

whether or not the motivational impairment effects seen as a result of FLX administration are

possible to reverse through pharmacological mechanisms.

Additionally, future directions involve evaluating possible combined effects between the

$5\text{-HT}_{1B}R$ as it interacts with others. In particular, systemic co-administration of 5-HT_{1B} and 5-HT_{1C} receptor agonists has been shown to decrease firing of DAergic neurons in the VTA with a

greater extent than agonism of $5\text{-HT}_{1B}R$ alone[16]. It is possible that the reverse could be true as

well, with 5-HT_{1C} and 5-HT_{1B} antagonism potentially resulting in a greater if not full reversal of

the FLX-induced behavioral challenge. The determination of involvement of particular 5-HT

receptors in mediating motivated behaviors has proven to be difficult, however, further research

will contribute to the discovery of possible therapeutic mechanisms to treat the most debilitating

symptoms of MDD.

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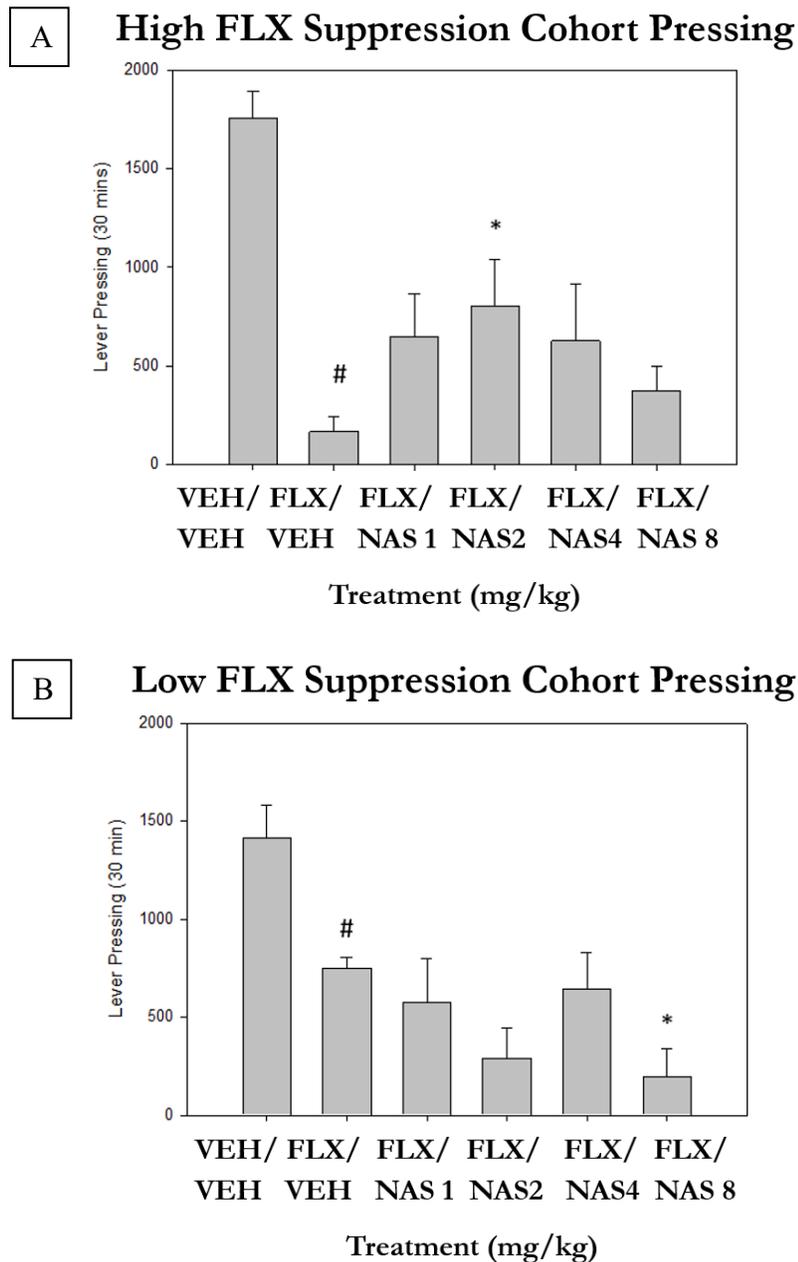
Figure 1

Figure 1. Mean (±SEM) number of lever presses for the high FLX suppression (1A) and low

FLX suppression (1B) cohorts following coadministration of FLX along with NAS, a selective 5-

$HT_{1B}R$ antagonist in a FR5/chow behavioral choice task. Doses of NAS ranged from 1.0 mg/kg

to 8 mg/kg. **1A**, FLX suppression paired comparisons from VEH/VEH to FLX/VEH was

significant [# denotes $F_{(1,25)}=33.255$ $p<0.01$] as was the partial reversal from FLX/VEH to FLX/NAS2 (denoted by *) [$F=4.580$ $p<0.05$]. **1B**, FLX suppression paired comparisons from VEH/VEH to FLX/VEH was significant [# denotes $F_{(1,20)}=7.125$ $p<0.025$] as was the attenuation from FLX/VEH to FLX/NAS8 (denoted by *) [$F_{(1,20)}=5.328$ $p<0.05$]. The quadratic trend in the high FLX suppression cohort is significant when assessing the interaction between the two groups [$F_{(1,9)}=6.921$ $p<0.05$, $p=0.027$].