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# Administration of the Y2 Receptor Agonist $PYY_{3-36}$ in Mice Induces Multiple Behavioral Changes Relevant to Schizophrenia

**Ulrike Stadlbauer*[,1], Wolfgang Langhans[1] and Urs Meyer[1]**

[1]*Physiology and Behavior Laboratory, Swiss Federal Institute of Technology (ETH) Zurich, Schwerzenbach, Switzerland*

Functional changes in neuropeptide Y (NPY) signaling at the Y2 receptor subtype have been widely implicated in stress-related neuropsychiatric illnesses such as depression and anxiety disorders. Altered Y2 receptor signaling may also play a role in the precipitation of behavioral and cognitive symptoms associated with schizophrenia. To seek preclinical evidence for this possibility, we explored the functional consequences of treatment with the selective Y2 receptor agonist $PYY_{3-36}$ using translational tests for the assessment of schizophrenia-relevant behavioral and cognitive deficits in mice. We found that acute systemic administration of $PYY_{3-36}$ at a low dose (1 μg/100 g body weight) or high dose (20 μg/100 g body weight) profoundly impaired social interaction without affecting innate anxiety. $PYY_{3-36}$ treatment at the high dose further led to a disruption of sensorimotor gating in the form of prepulse inhibition deficiency. This effect was fully antagonized by acute treatment with the preferential dopamine D2 receptor antagonist haloperidol, but not with clozapine. In addition, both doses of $PYY_{3-36}$ impaired selective associative learning in the latent inhibition paradigm and spatial working memory in a matching-to-position water maze test. The wide range of abnormalities induced by $PYY_{3-36}$ suggests that signaling at the Y2 subtype of NPY receptors is critical for a number of behavioral and cognitive functions, some of which are highly relevant to schizophrenia and related psychotic disorders. At least some of the behavioral deficits induced by augmentation of Y2 receptor signaling may involve increased dopaminergic activity.
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**Keywords:** neuropeptides; latent inhibition; cognition; dopamine; prepulse inhibition; social interaction

## INTRODUCTION

Neuropeptide Y (NPY) is a 36-amino-acid peptide that is widely distributed in the central nervous system (CNS) (Tatemoto *et al*, 1982). It has been recognized to regulate a number of behavioral and physiological functions, including eating behavior, energy balance, and cardiovascular functions (Brothers and Wahlestedt, 2010). In addition, NPY is pivotal for the homeostatic control of stress responses and critically modulates affective and emotional behaviors (Morales-Medina *et al*, 2010; Wu *et al*, 2011). Given its role in mood and affect, impaired NPY signaling has been repeatedly implicated in stress-related neuropsychiatric illnesses such as depression, post-traumatic stress disorders (PTSD), and anxiety disorders (Heilig, 2004).

Several lines of evidence implicate altered NPY signaling in schizophrenia, a chronic form of psychotic disorder characterized by multiple and coexisting behavioral dysfunctions (Tandon *et al*, 2009). For example, reduced NPY levels have been found in frontal cortical tissues of patients with schizophrenia (Ikeda *et al*, 2004; Kuromitsu *et al*, 2001) and schizoaffective disorder (Morris *et al*, 2009). As NPY is synthesized in subclasses of GABAergic interneurons (Vezzani and Sperk, 2004), the deficit in cortical NPY expression is congruent with the proposed GABAergic pathology in schizophrenia spectrum disorder (Hashimoto *et al*, 2008). Moreover, treatment with clinically effective antipsychotic drugs (APDs) increases NPY levels in schizophrenic patients (Nikisch *et al*, 2012; Obuchowicz *et al*, 2004).

The NPY system is further known to modulate central dopamine signaling that in turn has been widely implicated in the pathophysiology of psychotic symptoms (Howes and Kapur, 2009). Activation of the Y2 subtype of NPY receptors seems to assume a key role in mediating such dopaminergic effects. For example, selective Y2 receptor agonists have been shown to enhance the release of dopamine from striatal slices in rats (Adewale *et al*, 2007). Functional magnetic resonance imaging in humans has further demonstrated that administration of selective Y2 receptor agonists induce neuronal activation in mesolimbic dopamine pathways (Batterham *et al*, 2007). Even though the precise molecular mechanisms underlying these dopaminergic effects remain to be elucidated, they likely involve

*Correspondence: U Stadlbauer, Physiology and Behavior Laboratory, Swiss Federal Institute of Technology (ETH) Zurich, Schorenstrasse 16, Schwerzenbach 8603, Switzerland. Tel: +414 4655 7486, Fax: +414 4655 7206, E-mail: ulrike-stadlbauer@ethz.ch
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Y2-mediated autoinhibitory suppression of NPY release (King *et al*, 1999; Smith-White *et al*, 2001; Stanic *et al*, 2011)

Animal studies further suggest that altered Y2 receptor signaling has a number of behavioral consequences, including modulation of emotional, affective, and social behavior (Karl *et al*, 2010; Morales-Medina *et al*, 2012a; Redrobe *et al*, 2003, 2004). Characterization of the behavioral consequences resulting from Y2 receptor modulation, however, remains incomplete and warrants further extension to functional domains more directly implicated in schizophrenic disease. Therefore, the present study sought to evaluate the impact of acute Y2 receptor agonism on schizophrenia-relevant behavioral functions in mice.

We used a pharmacological approach exploring the behavioral effects of the peptide hormone $PYY_{3-36}$ (peptide tyrosine-tyrosine 3-36) that, together with NPY and pancreatic polypeptide (PP), forms the PP-fold family of peptides (Berglund *et al*, 2003). Following systemic $PYY_{3-36}$ administration, the peptide effectively crosses the blood–brain barrier (Nonaka *et al*, 2003) and acts as a selective Y2 receptor agonist (Dumont *et al*, 1994; Grandt *et al*, 1992, 1993; Keire *et al*, 2000, 2002). Treatment with $PYY_{3-36}$ inhibits the release and synthesis of NPY (Acuna-Goycolea and van den Pol, 2005; Batterham *et al*, 2002; Challis *et al*, 2003) in line with the autoinhibitory feed-back mechanism attributed to the Y2 receptor (Wu *et al*, 2011). In addition, exogenous administration of $PYY_{3-36}$ has been found to markedly potentiate potassium-evoked release of newly synthesized dopamine in the rat striatum (Adewale *et al*, 2007). Hence, $PYY_{3-36}$ is capable of inducing hyperdopaminergic effects that may facilitate the induction of psychosis-related behavioral abnormalities.

To study the effects of $PYY_{3-36}$ on schizophrenia-related behaviors, we implemented translational paradigms assessing a number of key neuropsychological and neurocognitive functions known to be impaired in schizophrenia and related disorders (Peleg-Raibstein *et al*, 2012). This included the examination of social interaction, central information processing in the form of sensorimotor gating, as well as selective associative learning and spatial working memory. In addition, we ascertained the effects of $PYY_{3-36}$ on anxiety-related behavior and evaluated the capacity of reference APDs to antagonize some of the anticipated behavioral deficits induced by $PYY_{3-36}$.

## MATERIALS AND METHODS

### Animals

Male C57BL6 mice were used throughout the study. They were obtained from Charles River (Charles River, Sulzfeld, Germany) at the age of 8–10 weeks. Testing began after 2 weeks of acclimatization to the new animal holding room that was temperature and humidity controlled ($21 \pm 2\,^{\circ}C$, $55 \pm 5\%$) with a 12-h light and 12-h dark cycle (lights off at 0700 h). All animals had *ad libitum* access to water and food (Kliba 3436, ProvimiKliba NAFAG, Kaiseraugst, Switzerland) and were kept in groups of 3–5 animals per cage throughout all experimentation. Behavioral testing was always carried out during the dark phase of the 12-h light and 12-h dark cycle. Some of the behavioral testing (elevated plus maze, social interaction, and water maze working memory tests) involved brief exposure of the animals to a well-lit room during the dark phase period. To minimize potential confounding factors associated with prolonged testing and/or drug administration, three different cohorts were used to accomplish all tests of interest (see Table 1).

**Table 1** Number of Cohorts and Animals in Each Cohort

| Cohort | Test | Dose of $PYY_{3-36}$ | | | $PYY_{3-36}$ injection test interval | Test duration |
|---|---|---|---|---|---|---|
| | | 0 μg/100 g (= VEH) | 1 μg/100 g (= PYY-1) | 20 μg/100 g (= PYY-20) | | |
| 1 | EPM | 9 | 9 | 8 | 15 min | 10 min |
| | SI | 9 | 9 | 8 | 15 min | 5 min |
| | PPI | 9 | 9 | 8 | 5 min | 40 min |
| 2 | *LI* | | | | 5 min (preexposure and conditioning); 15 min (CS test) | 75 min (preexposure and conditioning); 7.5 min (CS test) |
| | NPE | 7 | 7 | 7 | | |
| | PE | 7 | 7 | 7 | | |
| | WM | 11 | 10 | 10 | 15 min | 1–5 min |
| 3 | *PPI with APDs* | | | | | |
| | VEH/VEH | 11 | – | – | 5 min | 40 min |
| | PYY-20/VEH | – | – | 12 | 5 min | 40 min |
| | PYY-20/HAL | – | – | 9 | 5 min | 40 min |
| | PYY-20/CLZ | – | – | 8 | 5 min | 40 min |

The table outlines the number of mice used for each test/cohort as well as the injection test interval and test duration. Vehicle (VEH) or $PYY_{3-36}$ at the low dose (1 μg/100 g body weight = PYY-1) or high dose (20 μg/100 g body weight = PYY-20) were administered using different injection test intervals depending on the precise behavioral test in order to induce peak $PYY_{3-36}$ levels in the CNS during the time of testing. Animals in cohort 1 were repeatedly tested in the elevated plus maze (EPM) test, social interaction (SI) test, and prepulse inhibition (PPI) test with a washout period of at least 7 days between the individual tests. Animals in cohort 2 were first tested in the latent inhibition (LI) test and subsequently in the working memory (WM) test. Animals in cohort 3 were used to assess the effects of the antipsychotic drugs haloperidol (HAL) or clozapine (CLZ) against PPI disruption induced by the high dose of $PYY_{3-36}$ (PYY-20).

All procedures were approved by the Cantonal Veterinary Office of Zurich and are in agreement with the principles of laboratory animal care in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 86-23, revised 1985). All efforts were made to minimize the number of animals used and their suffering.

### $PYY_{3-36}$ Treatment

$PYY_{3-36}$ (Bachem FBC0047; Bachem, Bubendorf, Switzerland) was dissolved in phosphate-buffered saline (PBS) containing 1% of bovine serum albumin (BSA) and administered at a dose of 1 or 20 μg/100g body weight (BW; referred to as PYY-1 and PYY-20, respectively). These doses were selected based on previous studies using $PYY_{3-36}$ in rodents reflecting $PYY_{3-36}$ administration at low (PYY-1) and high (PYY-20) intensities (Batterham *et al*, 2002; Challis *et al*, 2003; Pittner *et al*, 2004; Vrang *et al*, 2006). Vehicle-treated (VEH) animals received PBS only. All solutions were freshly prepared on the day of testing and administered intraperitoneally (i.p.) using an injection volume of 5 ml/kg. The injection-test intervals were adjusted for each behavioral test (Table 1) in order to induce peak $PYY_{3-36}$ levels in the CNS during the time of testing (Nonaka *et al*, 2003). To minimize the number of animals required to complete all tests of interest, mice were repeatedly administered with $PYY_{3-36}$ for a maximum of 3 times per animal (Table 1). A wash-out period of at least 7 days was implemented between individual $PYY_{3-36}$ injections/tests to avoid possible confounds arising from tolerance to the peptide (Reidelberger *et al*, 2008; van den Hoek *et al*, 2007).

### Elevated Plus Maze Test

Anxiety-related behavior was studied using a standard elevated plus maze test as fully described in the Supplementary Materials and Methods. The rationale for this test was to rule out the possibility that $PYY_{3-36}$ administration at the selected doses changes anxiety-like behavior that could be a confounding factor for some of the other behavioral tests of primary interest.

### Social Interaction Test

Deficits in social interaction are often noted in patients with schizophrenia (Foussias and Remington, 2010) and in translational rodent models of the disorder (Peleg-Raibstein *et al*, 2012). Here, we assessed the effects of $PYY_{3-36}$ on social interaction using a social approach test in a modified Y-maze as established before (Vuillermot *et al*, 2011). Social interaction was assessed by analyzing the relative exploration time between an unfamiliar congenic mouse and an inanimate dummy object. A detailed description of the test apparatus and procedure is given in the Supplementary Materials and Methods.

### Prepulse Inhibition Test

Sensorimotor gating was assessed using the paradigm of prepulse inhibition (PPI) of the acoustic startle reflex. PPI of the acoustic startle reflex refers to the reduction of startle reaction in response to a startle-eliciting pulse stimulus when it is shortly preceded by a weak prepulse stimulus (Hoffman and Searle, 1965). The PPI test was performed in relation to human studies documenting PPI deficits in schizophrenia and related disorders (Braff *et al*, 2001). A thoroughly validated protocol and test procedures were used for the PPI test as described in the Supplementary Materials and Methods.

### Latent Inhibition Test

Selective associative learning was measured using the paradigm of latent inhibition (LI), in which non-reinforced preexposures to a to-be-conditioned stimulus (CS) retards subsequent conditioning between the same CS and the unconditioned stimulus (US) (Lubow, 2005). LI is considered to index an organism's capacity to ignore irrelevant stimuli and is disrupted in at least a subset of schizophrenic patients, especially in acutely ill subjects experiencing marked positive symptoms (Lubow, 2005; Weiner, 2003; Weiner and Arad, 2009). LI was assessed in a conditioned freezing paradigm, in which a tone served as the CS and electric foot shock as the US (see Supplementary Materials and Methods).

### Spatial Working Memory Test

Working memory is a special short-term memory buffer used to hold relevant information temporarily active in order to guide on-going behavior (Baddeley, 2003), and its disruption is a cardinal cognitive symptom in schizophrenia (Goldman-Rakic, 1994). Against this background, we evaluated the effects of $PYY_{3-36}$ on spatial working memory using an established matching-to-position working memory paradigm in the Morris water maze. A detailed description of the test apparatus and experimental protocol is given in the Supplementary Materials and Methods.

### Antipsychotic Drug Administration

We examined whether acute pretreatment with the reference typical and atypical APDs haloperidol (HAL) and clozapine (CLZ), respectively, would be effective in antagonizing the anticipated behavioral deficits induced by $PYY_{3-36}$. For this purpose, we selected PPI because HAL and CLZ have been widely documented to be capable of restoring PPI deficits in a variety of translational rodent models of schizophrenia and related disorders (Geyer *et al*, 2001; Swerdlow *et al*, 2008). HAL (5 mg HAL/ml of solvent containing minimal amounts of lactic acid; Janssen-Cilag, Baar, Switzerland) was diluted with 0.9% sterile NaCl. CLZ (Novartis, Basel, Switzerland) was first dissolved in 0.1 N hydrochloric acid (HCl) in 0.9% sterile NaCl and then titrated with $Na_2CO_3$ to obtain a pH of ~5.5. HAL and CLZ were administered at doses of 0.2 and 1.5 mg/kg, respectively, based on previous PPI testing in C57BL6 mice (Russig *et al*, 2004; Singer and Yee, 2012; Yee *et al*, 2005). Vehicle-treated animals received either 0.9% sterile NaCl solution alone (HAL experiment) or 0.1 N HCl in 0.9% sterile NaCl solution with appropriate amounts of $Na_2CO_3$, pH ~5.5 (CLZ experiment). All solutions were freshly prepared on the day of testing and administered i.p. using an injection volume of 5 ml/kg. All solutions were injected

45 min before PPI testing according to established protocols (Singer and Yee, 2012; Yee *et al*, 2005).

### Statistical Analyses

All data were analyzed by parametric analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) *post hoc* comparisons or restricted ANOVAs as fully described in the Supplementary Materials and Methods. The analysis of the PPI data was supplemented with additional analyses of covariance (ANCOVA). Statistical significance was set at $P<0.05$. All statistical analyses were performed using the statistical software StatView (version 5.0) and SPSS for Windows (version 20).

## RESULTS

### $PYY_{3-36}$ Treatment Impairs Social Interaction without Affecting Innate Anxiety

First, we evaluated the effects of $PYY_{3-36}$ on innate anxiety-like and social behavior. As summarized in Figure 1a, neither the low (1 μg/100 g BW; = PYY-1) nor the high (20 μg/100 g BW; = PPY-20) dose of $PYY_{3-36}$ affected the percent open arm time or percent open arm frequency in the elevated plus maze test of innate anxiety. General locomotor activity as indexed by the total distance moved in the elevated plus maze was also comparable among the three experimental groups (Figure 1a).

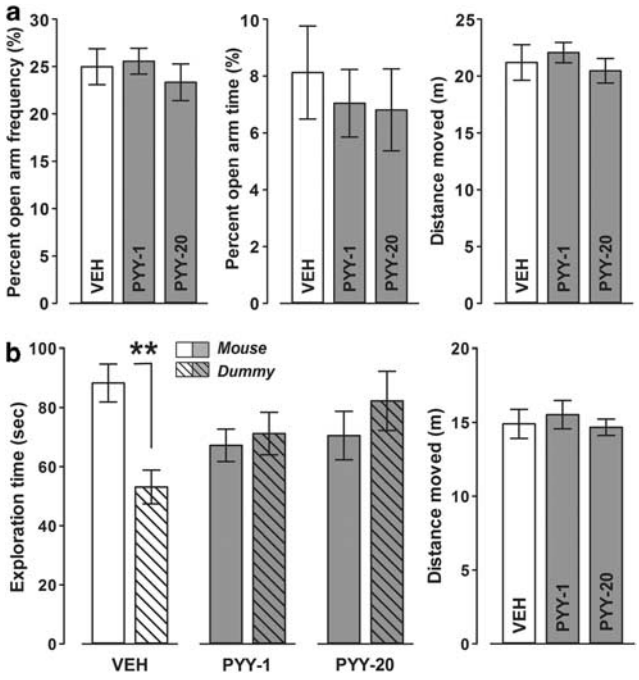


**Figure 1** Effects of $PYY_{3-36}$ treatment on anxiety-related and social behavior. Mice were injected with vehicle (VEH) or $PYY_{3-36}$ at low dose (1 μg/100 g body weight (PYY-1)) or high dose (20 μg/100 g body weight (PYY-20)) before behavioral examination. (a) The bar plots depict percent open arm frequencies, percent open arm time, and distance moved in the elevated plus maze test of anxiety-related behavior. (b) The bar plots show the relative exploration time between an unfamiliar congenic mouse ('mouse') and an inanimate dummy ('dummy') for the VEH, PYY-1, and PYY-20 groups, as well as the corresponding distances moved in the social interaction test. $^{**}P<0.01$, reflecting the significant main effect of object in the VEH group. All values are means ± SEM.

In the social interaction test, the relative exploration time between an unfamiliar congenic mouse and an inanimate dummy object was used to index social interaction. VEH-treated mice displayed a clear preference toward the unfamiliar mouse, indicating intact social interaction (Figure 1b). In contrast, animals exposed to the low (PYY-1) or high (PYY-20) dose of $PYY_{3-36}$ did not show such a preference (Figure 1b). These patterns of results led to a significant interaction between PYY treatment and object ($F(2,23)=5.62$, $P<0.05$). Additional analyses restricted to each experimental group confirmed the preference of VEH-treated control animals toward the live mouse relative to the dummy object ($F(1,8)=18.07$, $P<0.01$), whereas there was no main effect of object in the $PYY_{3-36}$-exposed animals. Consistent with the outcomes in the elevated plus maze test (Figure 1a), the $PYY_{3-36}$ treatments did not affect locomotor activity as measured by the distance moved during the social interaction test (Figure 1b).

### $PYY_{3-36}$ Treatment Impairs Central Information Processing

We further explored the effects of $PYY_{3-36}$ on central information processing. First, we used the paradigm of PPI of the acoustic startle reflex to assess sensorimotor gating. As expected, the magnitude of % PPI increased with increasing prepulse intensities in all animals, leading to a significant main effect of prepulse levels ($F(4,92)=30.78$, $P<0.001$). $PYY_{3-36}$ administration also affected % PPI scores: animals treated with the high dose of $PYY_{3-36}$ (PYY-20) exhibited an ~30% reduction in % PPI compared with VEH-treated control animals and animals exposed to the low dose of $PYY_{3-36}$ (PYY-1) (Figure 2a), leading to a significant main effect of PYY treatment ($F(2,23)=5.35$, $P<0.01$). Subsequent *post hoc* comparisons confirmed the significant differences in % PPI between VEH and PYY-20 mice ($P<0.01$) and between PYY-1 and PYY-20 mice ($P<0.05$). Administration of $PYY_{3-36}$ did not affect the animals' reactivity to prepulse-alone trials (data not shown). Furthermore, $PYY_{3-36}$ treatment did not significantly affect the animals' startle reaction to pulse-alone trials, even though a tendency toward reduced startle reactivity was observed following $PYY_{3-36}$ administration. The means ± SEM of reactions to pulse-alone trials were 196.07 ± 27.83 for VEH animals, 158.38 ± 22.47 for PYY-1 animals, and 126.24 ± 17.50 for PYY-20 animals.

We performed a series of additional analyses to further ascertain whether the $PYY_{3-36}$-induced variations in startle reactivity might confound the interpretation of the % PPI data. First, we conducted an ANCOVA to test whether the variations of startle reactivity could account for the effect of PYY on % PPI. To this end, ANCOVA of mean % PPI was conducted with startle reactivity as the covariate. This additional analysis showed that the main effect of PYY on % PPI remained highly significant ($P<0.01$), whereas the covariate term of pulse reactivity failed to attain statistical significance ($P=0.12$). Furthermore, we reanalyzed the PPI data in a subgroup of animals, for which we matched startle reactivity as far as possible (see Supplementary Figure 1). Under these conditions, the (nonsignificant) group differences

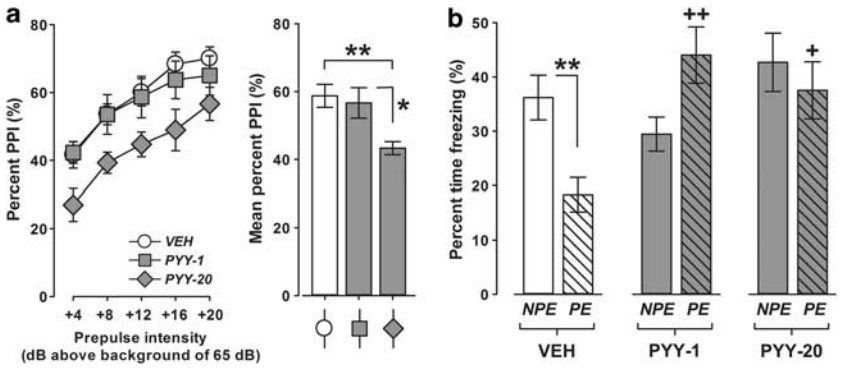


**Figure 2** Effects of PYY$_{3-36}$ treatment on central information processing. Mice were injected with vehicle (VEH) or PYY$_{3-36}$ at low dose (1 μg/100 g body weight (PYY-1)) or high dose (20 μg/100 g body weight (PYY-20)) before behavioral examination. (a) The line plot shows percent prepulse inhibition (% PPI) as a function of different prepulse intensities in the test of PPI of the acoustic startle reflex. The bar plot depicts the mean % PPI across all prepulse levels tested. $^{*}P<0.05$ and $^{**}P<0.01$. (b) The bar plots show percent time freezing in non-preexposed (NPE) and preexposed (PE) subjects during the tone CS test of the latent inhibition task. $^{**}P<0.01$, reflecting the significant difference between NPE and PE subjects (ie, LI) in the VEH group; $^{+}P<0.05$ and $^{++}P<0.01$, reflecting the significant difference between the VEH/PE and PYY-20/PE groups and between the VEH/PE and PYY-1/PE groups, respectively. All values are means ± SEM.

in startle reactivity became minimal (Supplementary Figure 1). At the same time, however, the differences in % PPI between VEH animals and PYY-20 animals remained statistically significant (Supplementary Figure 1).

To obtain an additional behavioral readout of central information processing, we measured the acquisition (conditioning phase) and expression (CS test phase) of the LI effect in Pavlovian fear conditioning. During the initial conditioning phase of the test, the amount of % time freezing generally increased as a function of successive CS/US pairings. As expected, prior exposure to the CS before conditioning attenuated the development of the conditioned freezing response. This effect was most notable in the first two conditioning trials, as supported by the significant main effect of trials ($F(2,72)=132.87$, $P<0.001$) and its interaction with preexposure ($F(2,72)=11.98$, $P<0.001$). PYY$_{3-36}$ treatment did not significantly influence the development of conditioned freezing, and no main effect or interaction involving the between-subject factor of PYY treatment attained statistical significance. The overall means ± SEM of % time freezing across the successive CS/US trials was 13.18 ± 2.48 (trial 1), 40.48 ± 3.94 (trial 2), and 34.92 ± 3.64 (trial 3) for NPE subjects; and 1.27 ± 0.96 (trial 1), 29.05 ± 3.74 (trial 2), and 40.79 ± 3.98 (trial 3) for PE subjects.

The expression of conditioned fear toward the tone CS was evaluated in NPE and PE subjects 24 h after conditioning. As expected, VEH-treated mice expressed a noticeable LI effect during the 90-s tone CS test phase. This LI effect was evident by an increase in % time freezing displayed by VEH-treated NPE subjects relative to VEH-treated PE subjects (Figure 2b). Animals exposed to the low (PYY-1) or high (PYY-20) dose of PYY$_{3-36}$ did not show such a LI effect (Figure 2b), leading to a significant interaction between PYY treatment and preexposure ($F(2,36)=6.26$, $P<0.01$). Subsequent analyses restricted to each treatment group confirmed the significant difference between NPE and PE subjects in the VEH group ($F(1,12)=11.80$, $P<0.01$) but not in the PYY$_{3-36}$ groups (F's $<1$). Additional analyses restricted to the two preexposure conditions (ie, NPE and PE) were then performed in order to evaluate whether the PYY$_{3-36}$-induced LI disruption would be mediated via NPE or PE subjects. Whereas the analysis restricted to NPE subjects did not reveal any significant effects, the main effect of PYY treatment attained statistical significance in the analysis restricted to PE subjects ($F(2,18)=6.74$, $P<0.01$). Subsequent *post hoc* comparisons conducted for PE subjects further revealed a significant difference between the VEH and PYY-1 ($P<0.01$) and between the VEH and PYY-20 groups ($P<0.05$).

## PYY$_{3-36}$ Treatment Impairs Spatial Working Memory

We extended the assessment of the cognitive consequences of PYY$_{3-36}$ administration to a matching-to-position test of spatial working memory in the Morris water maze. Following initial habituation training, all animals were first treated with VEH solution only for two consecutive days of working memory testing (VEH injection phase). This initial phase served to stabilize the animals' performance after exposure to additional stress by injections. Animals that exhibited excessive floating or did not display any improvement from trial 1 to 2 during the VEH injection phase were excluded from the subsequent test phase, which took place 1 day after the last VEH injection.

As summarized in Figure 3a, the implementation of the initial VEH injection phase allowed us to establish three groups of animals with highly comparable working memory performance following VEH injection. Indeed, the analysis of the latency (s, ln-transformed) to locate the submerged platform during VEH injection phase only revealed a highly significant main effect of trials ($F(1,28)=99.79$, $P<0.001$). In the subsequent test phase, VEH-exposed animals still demonstrated a significant reduction in the latency to find the submerged platform in trial 2 relative to trial 1, indicating intact working memory (Figure 3b and c). In marked contrast, animals receiving either the low (PYY-1) or high (PYY-20) dose of PYY$_{3-36}$ no longer showed a similar improvement from trial 1 to trial 2 (Figure 3b and c). The analysis of latency (s, ln-transformed) during the test day revealed a significant interaction between PYY treatment and trials ($F(2,28)=3.89$, $P<0.05$). Subsequent

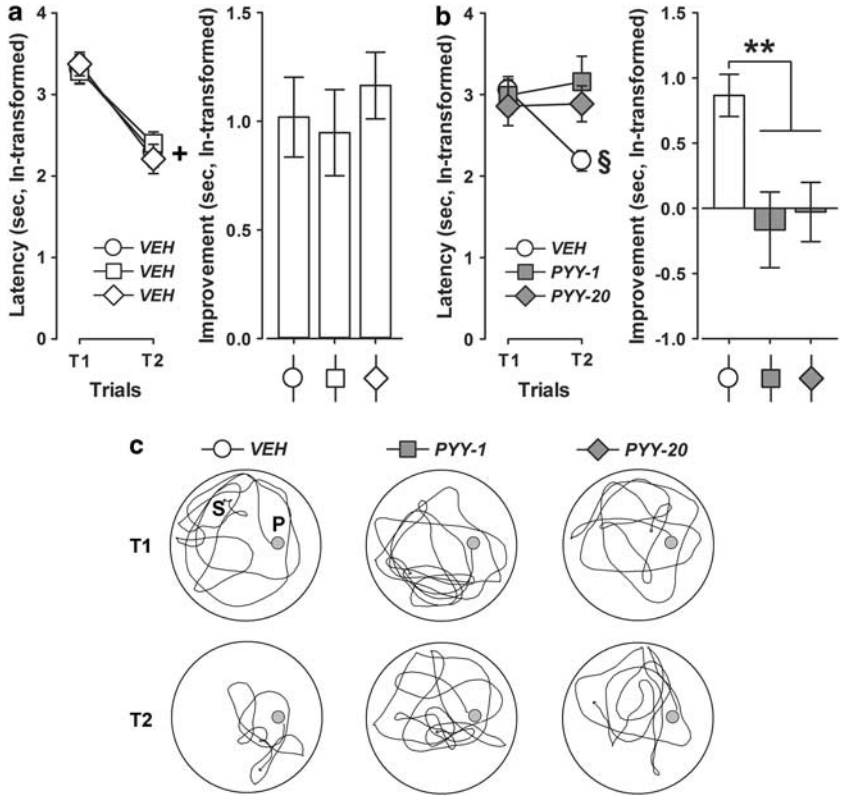


**Figure 3** Effects of $PYY_{3-36}$ treatment on spatial working memory in a matching-to-position water maze test. The line plots show the latency (s, ln-transformed) to find the hidden platform in trial 1 (T1) and T2 of the working memory test, and the bar plots depict the improvement in these measures from T1 to T2. (a) All mice were first injected with vehicle (VEH) solution only before working memory assessment. $^{+}P<0.001$, reflecting the significant main effect of trials. All values are means ± SEM. (b) In the subsequent test phase, one third of animals received VEH again and the remaining animals were injected with $PYY_{3-36}$ at low dose (1 μg/100 g body weight (PYY-1)) or high dose (20 μg/100 g body weight (PYY-20)) before working memory assessment. $^{\S}P<0.001$, reflecting the significant main effect of trials in the VEH group; $^{**}P<0.01$, based on *post hoc* comparison of the improvement in latencies. All values are means ± SEM. (c) The drawings illustrate computer-generated search path of representative VEH, PYY-1, and PYY-20 animals in T1 and T2 of the working memory test. S, Starting position; P, position of the hidden platform.

analyses restricted to each treatment group confirmed the presence of a highly significant trial effect in VEH-treated animals ($F(1, 10) = 28.73$, $P<0.001$), but not in $PYY_{3-36}$-exposed animals (F's $<0.2$).

### Acute Haloperidol Treatment Antagonizes the $PYY_{3-36}$-Induced Prepulse Inhibition Deficits

We examined the efficacy of acute HAL or CLZ pretreatment to antagonize the $PYY_{3-36}$-induced PPI disruption. Consistent with our previous findings (Figure 2a), $PYY_{3-36}$/VEH animals displayed a significant reduction in % PPI compared with VEH/VEH control animals (Figure 4a). Pretreatment with HAL but not with CLZ fully reversed the $PYY_{3-36}$-induced PPI disruption (Figure 4a). The analysis of % PPI revealed a significant main effect of group ($F(3, 37) = 8.18$, $P<0.001$). Subsequent *post hoc* comparisons confirmed the significant difference between VEH/VEH and $PYY_{3-36}$/VEH ($P<0.01$) as well as between $PYY_{3-36}$/VEH and $PYY_{3-36}$/HAL ($P<0.01$) animals. In addition, the $PYY_{3-36}$/CLZ group differed significantly from the VEH/VEH ($P<0.05$) and $PYY_{3-36}$/HAL ($P<0.01$) groups (Figure 4a).

$PYY_{3-36}$/CLZ-treated animals tended to exhibit reduced (but nonsignificant) startle reactivity (Figure 4b). The analysis of the reactivity to pulse-alone trials did not yield any significant effects. Nevertheless, we performed an additional ANCOVA of mean % PPI with startle reactivity as the covariate in order to test whether the (nonsignificant) variations of startle reactivity could account for the group differences in % PPI. This additional analysis revealed a highly significant main effect of group ($P<0.001$), whereas the covariate term of startle reactivity failed to reach statistical significance ($P=0.77$).

The $PYY_{3-36}$/CLZ group showed significantly increased reactivity to prepulse-alone trials (Figure 4c). This effect was largely evident across all prepulse levels, leading to a significant main effect of group in the analysis of prepulse-induced reactivity ($F(3, 37) = 3.72$, $P<0.05$). Subsequent *post hoc* comparisons confirmed the significant difference between $PYY_{3-36}$/CLZ animals and all other groups ($P<0.01$).

## DISCUSSION

Our study demonstrates that acute systemic treatment with the Y2 receptor agonist $PYY_{3-36}$ in mice leads to functional alterations in multiple behavioral domains, including social interaction, sensorimotor gating, selective associative

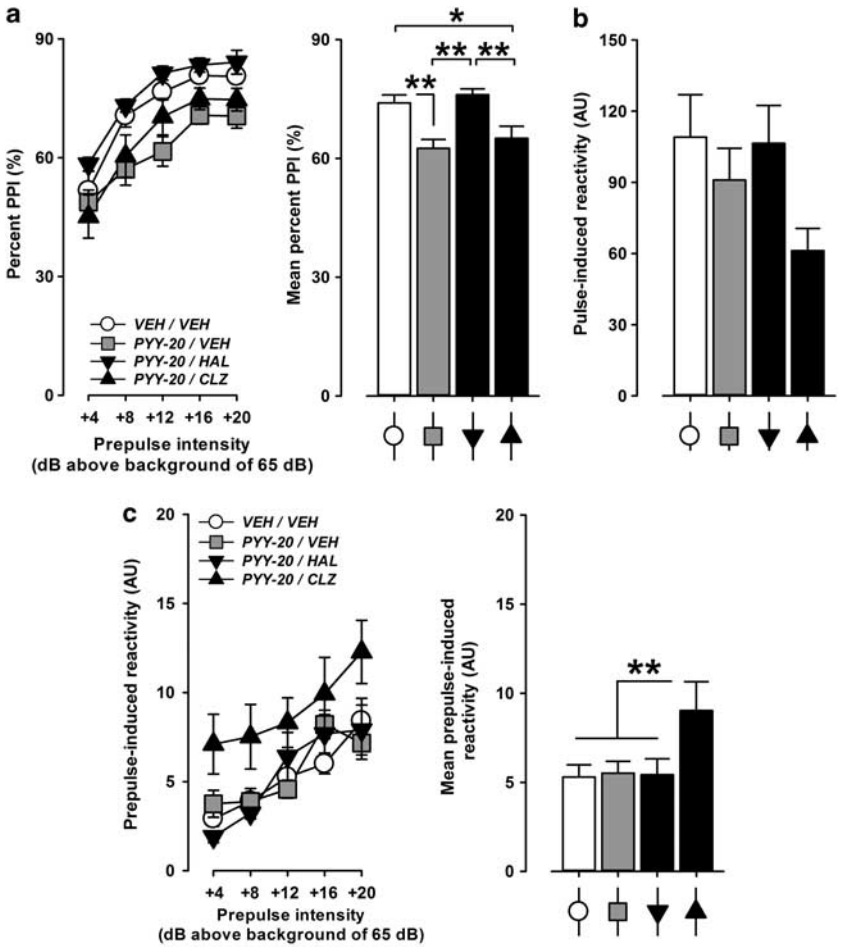


**Figure 4** Effects of antipsychotic drugs on PYY$_{3-36}$-induced prepulse inhibition deficits. Mice were pretreated with haloperidol (HAL, 0.2 mg/kg), clozapine (CLZ, 1.5 mg/kg), or corresponding vehicle (VEH) solution before administration of PYY$_{3-36}$ (20 μg/100 g body weight), and the effects were compared with control mice receiving appropriate VEH solution twice. (a) The line plot shows percent prepulse inhibition (% PPI) as a function of different prepulse intensities, and the bar plot depicts the mean % PPI across all prepulse levels tested. $^{*}P<0.05$ and $^{**}P<0.01$. (b) The bar plot shows the reactivity to pulse-alone trials (in arbitrary units (AU)). (c) The line plot depicts the reactivity to prepulse-alone trials as a function of different prepulse intensities, and the bar plot depicts the mean prepulse-induced reactivity across all prepulse levels tested. $^{**}P<0.01$. All values are means ± SEM.

learning, and spatial working memory. The spectrum of abnormalities induced by PYY$_{3-36}$ suggests that signaling at the Y2 subtype of NPY receptors is critical for a number of behavioral and cognitive functions that go beyond those previously implicated in eating behavior and energy balance (Brothers and Wahlestedt, 2010). Recent evidence further indicates that altered Y2 receptor signaling may play an important role in precipitating stress-related and depression-like behavior (Heilig, 2004; Morales-Medina *et al*, 2010; Wu *et al*, 2011). Our data provide an important addition to this literature by emphasizing that acute Y2 receptor agonism modulates behavioral and cognitive functions implicated in schizophrenia and related psychotic disorders.

Deficits in PPI have been widely (but not exclusively) documented in schizophrenic patients (Braff *et al*, 2001; Swerdlow *et al*, 2008), and so have impairments in (spatial) working memory (Goldman-Rakic, 1994). Moreover, LI is disrupted in at least a subset of schizophrenic patients, especially in acutely ill subjects experiencing marked positive symptoms (Lubow, 2005; Weiner, 2003; Weiner and Arad, 2009). Finally, social interaction deficits are prominent in schizophrenic patients with marked negative symptoms (Foussias and Remington, 2010) and are also a cardinal feature of related neurodevelopmental disorders, especially autism (Crawley, 2007). Overall, the wide range of behavioral and cognitive abnormalities induced by PYY$_{3-36}$ administration suggests that this peptide treatment is capable of mimicking functional changes relevant to the positive/negative symptom dichotomy of schizophrenia as well as the neurocognitive symptoms associated with the disease (Peleg-Raibstein *et al*, 2012).

The PYY$_{3-36}$-induced PPI deficits were associated with a nonsignificant trend toward reduced startle reactivity. It has been widely acknowledged that concomitant changes in startle reactivity might complicate the interpretation of whether or not differences in % PPI might reflect a genuine deficit in sensorimotor gating (Swerdlow *et al*, 2000). The outcomes of the additional ANCOVAs of mean % PPI with startle reactivity as the covariate suggested that the (nonsignificant) variations of startle reactivity could not account for the group differences in % PPI. Furthermore, significant differences in % PPI persisted when the data were analyzed in a subgroup of animals matched for

minimal variations in startle reactivity. We believe that these findings can be taken to support our interpretation that PYY at the higher dose is indeed capable of affecting the processes of sensorimotor gating, even though we cannot fully exclude the possibility that these effects on gating may, to some extent, be associated with concomitant effects on the startle reactivity *per se*.

Our study further shows that pretreatment with the typical APD HAL fully antagonizes the $PYY_{3-36}$-induced deficits in PPI. In contrast, the atypical APD CLZ failed to increase PPI scores in $PYY_{3-36}$-treated animals, even though it significantly increased prepulse-induced reactivity and led to a tendency toward decreased startle reactivity. HAL is a preferential dopamine D2 receptor antagonist, whereas the neurochemical profile of CLZ involves modulation of various neurotransmitter systems (Miyamoto *et al*, 2012). It thus appears that the $PYY_{3-36}$-induced disruption of PPI may, at least in part, be explained by increased dopaminergic signaling at dopamine D2 receptors (van den Buuse, 2010). This hypothesis fits well with previous findings in rats showing that administration of $PYY_{3-36}$ markedly potentiates potassium-evoked release of dopamine in the striatum (Adewale *et al*, 2007). It will be interesting to further explore to what extent other behavioral or cognitive abnormalities induced by $PYY_{3-36}$ involve similar hyperdopaminergic mechanisms. One clear limitation of the present study is that we evaluated the efficacy of APD treatment to restore $PYY_{3-36}$-induced PPI impairments using acute administration of one dose of HAL and CLZ only. Hence, our data cannot exclude the possibility that compounds with a low potency to antagonize dopamine receptors (such as CLZ) may be similarly effective in restoring the $PYY_{3-36}$-induced PPI impairments when administered chronically.

The $PYY_{3-36}$-induced deficits in social interaction and sensorimotor gating complement and corroborate the recent findings by Karl *et al* (2010) showing increased social behavior and significantly higher PPI in genetically modified mice lacking the Y2 receptor. Furthermore, the emergence of impaired social interaction following $PYY_{3-36}$ treatment is in agreement with the effects of the Y2 receptor antagonist BIIE0246 that has been shown to increase active contacts in a rat social interaction test (Morales-Medina *et al*, 2012a). The expressions of startle responses (and consequently the amount of PPI) and social interaction are, to some degree, influenced by innate and/or conditioned forms of anxiety (Brodkin, 2007; Groenink *et al*, 2008). Here, we did not reveal any significant effects of $PYY_{3-36}$ on anxiety-related behavior in the elevated plus maze test. Therefore, the $PYY_{3-36}$-induced deficits in social interaction and PPI are unlikely to be confounded by differences in anxiety-like behavior. The dissociation of these effects is consistent with pharmacological studies revealing significant influences of Y2 receptor antagonism on social behavior in the absence of concomitant effects on anxiety-like behavior (Morales-Medina *et al*, 2012a, b).

Another main finding of our study is that treatment with the Y2 receptor agonist $PYY_{3-36}$ fully abolished the LI effect. LI is a selective associative learning procedure, in which previous repeated preexposures to the CS reduce the development and/or expression of the CR following explicit pairings between the same CS and the US. In neuropsychological terms, LI is considered to index an organism's capacity to ignore irrelevant stimuli (Lubow, 2005; Weiner, 2003). Animals treated with $PYY_{3-36}$ did not show this LI effect. Interestingly, the $PYY_{3-36}$-induced loss of LI was clearly attributable to abnormally increased responding in PE subjects. $PYY_{3-36}$ thus abolished the efficacy of repeated CS preexposures to reduce the expression of the CR and, consequently, $PYY_{3-36}$-treated PE mice did not display the typical reduction in the CR as seen in VEH-treated PE animals. One implication from these findings is that the $PYY_{3-36}$-induced disruption of LI does not simply reflect general deficits in classical conditioning *per se*, but instead it readily mirrors the changes in the attentional processes regulating the expression of LI (Lubow, 2005; Weiner, 2003).

Significant cognitive consequences of Y2 receptor stimulation by $PYY_{3-36}$ were also evident in the test of spatial working memory. Even though Y2 receptor signaling has been implicated in long-term spatial memory before (Dos Santos *et al*, 2013; Redrobe *et al*, 2004), our data are the first to emphasize its role in working memory. Working memory is often conceptualized as a special short-term memory buffer used to hold relevant information temporarily active in order to guide on-going behavior (Baddeley, 2003). Successful performance in spatial working memory tests depends on a variety of factors. First, the test subject is required to allocate appropriate amounts of attention to the prevailing spatial environment, both during the initial acquisition trial (trial 1) and subsequent test trial (trial 2). Second, the subject needs to retrieve the relevant short-term spatial information based on its previous action (trial 1) in order to effectively fulfill the task in the test trial (trial 2). This cognitive demand is further dependent on the amount of experienced proactive interference, which occurs when processing on one trial negatively affects performance on a subsequent trial (Hartshorne, 2008). Our study fails to delineate the individual neurocognitive processes that contributed to the manifestation of spatial working memory deficits in $PYY_{3-36}$-treated animals. It seems feasible, however, that attentional deficits may play a role in this context. Indeed, $PYY_{3-36}$-treated animals displayed marked deficits in the LI test, which in turn is often used to index (selective) attentional abnormalities in translational rodent models and clinical conditions (Lubow, 2005; Weiner, 2003).

In conclusion, this study provides novel pharmacological evidence for a role of the Y2 receptor in modulating behavioral and cognitive functions relevant to schizophrenic disease. Even though widely recognized in stress-related affective disorders (Heilig, 2004; Morales-Medina *et al*, 2010; Wu *et al*, 2011), the role of altered Y2 receptor signaling has received somewhat scant attention in the context of schizophrenia. Future studies will be needed to further identify the precise neurochemical mechanisms underlying the behavioral and cognitive consequences of augmented Y2 receptor activation. Such attempts should also include studies aiming to reverse the $PYY_{3-36}$-induced deficits via localized manipulations targeting specific neurotransmitter systems and brain areas. Efforts toward this direction may help to establish improved therapeutic strategies for the treatment of schizophrenia-relevant behavioral and cognitive deficits that are associated with

altered central actions of NPY and downstream signaling at Y2 receptors.

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The authors declare no conflict of interest.

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