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Histamine H3 receptor regulates sensorimotor gating and dopaminergic signaling in the striatum

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Running Title:

H3R regulates sensorimotor gating and dopaminergic signaling

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Abbreviations: DAT, dopamine transporter; D1R, dopamine D1 receptor; D2R, dopamine D2 receptor; ERK1/2, extracellular signal-regulated kinase 1/2; GTS, Gilles de la Tourette syndrome; HDC, histidine decarboxylase, H1R, histamine H1 receptor; H3R, histamine H3 receptor; KO, knockout; PPI, prepulse inhibition; TH, tyrosine hydroxylase.

Abstract

The brain histamine system has been implicated in regulation of sensorimotor gating deficits and in Gilles de la Tourette syndrome (GTS). Histamine also regulates alcohol reward and consumption via H3 receptor (H3R), possibly through an interaction with the brain dopaminergic system. Here we identified the histaminergic mechanism of sensorimotor gating and the role of histamine H3R in the regulation of dopaminergic signaling. We found that H3R knockout mice displayed impaired prepulse inhibition (PPI) indicating deficiency in sensorimotor gating. H1R knockout and HDC knockout mice had similar PPI as their controls. Dopaminergic drugs increased PPI of H3R knockout mice to the same level as in control mice suggesting that changes in dopamine receptors might underlie deficient PPI response when H3R is lacking. Striatal D1R mRNA level was lower and D1 and D2 receptor-mediated activation of extracellular signal-regulated kinase 1/2 (ERK1/2) was absent in the striatum of H3R knockout mice suggesting that H3R is essential for the dopamine receptor-mediated signaling. In conclusion, these findings demonstrate that H3R is an important regulator of sensorimotor gating and the lack of H3R significantly modifies striatal dopaminergic signaling. These data support the usefulness of H3R ligands in neuropsychiatric disorders with pre-attentional deficits and disturbances in dopaminergic signaling.

Introduction

Prepulse inhibition of the startle reflex (PPI) is an operational measure of sensorimotor gating, in which the motor response to an abrupt, intense stimulus is inhibited by a preceding weak stimulus (Swerdlow, 2013). Impaired PPI has been observed in several neurological disorders, including schizophrenia, Gilles de la Tourette syndrome (GTS) and Huntington's disease. Alterations in the brain histaminergic system in schizophrenic and Huntington's disease patients suggest a role for histamine in the pathophysiology of these diseases (Goodchild et al., 1999; Prell et al., 1995). Genetic evidence has also indicated a dysfunction of histaminergic system in GTS: a highly penetrant nonsense mutation W317X in histidine decarboxylase (HDC) gene was found in a pedigree of a father and eight children with GTS (Ercan-Sencicek et al., 2010). Further, a recent study reported impaired PPI in both mice and humans carrying HDC mutations (Castellan Baldan et al., 2014).

Dopamine modulates e.g. motor and cognitive functions but is also a key determinant of PPI (Swerdlow, 2013). Recent *in vitro* studies have shown that dopamine D1 and D2 receptors (D1Rs and D2Rs) interact with the brain histamine system by forming receptor heterodimers with the histamine 3 receptor (H3R) (Ferrada et al., 2008; Ferrada et al., 2009; Moreno et al., 2011). The striatum is rich in these receptor types suggesting that mediation of PPI, movements and cognitive functions by dopaminergic neurotransmission are regulated by the brain histaminergic system. Histaminergic neurons in the tuberomamillary nucleus of the posterior hypothalamus send projections throughout the brain, including the striatum and cortex (Panula et al., 1989), important areas for sensorimotor gating. The effects of neuronal histamine are mediated via H1, H2 and H3 receptors (H1R, H2R, and H3R, respectively). H1R and H2R are postsynaptic receptors whereas H3R is present at both pre- and postsynaptic sites (Panula and Nuutinen, 2013). At histaminergic terminals H3R regulates the release of histamine but it is also expressed on other neurons and regulates the release of e.g. glutamate and acetylcholine.

Here, our aim was to first clarify whether histamine *per se* or a specific histamine receptor is responsible for regulation of sensorimotor gating by using HDC KO, H1R KO and H3R KO mice. After identifying H3R as a key element and necessary for normal PPI we examined the effect of selected dopaminergic drugs on the locomotor activity of H3R KO mice. We then studied whether signal transduction in the prefrontal cortex (PFC) and striatum after systemically administrated dopaminergic drugs is affected by the loss of H3R. The mRNA levels of different key elements of the dopaminergic system were also measured.

Materials and Methods

Animals

H3R knockout (H3R KO) mice were originally supplied by Janssen Research & Development, LLC (La Jolla, CA, USA) and generated on a background of 129/Ola and C57BL/6J mice (Kuhne et al., 2011). Selective backcrossing was conducted at Janssen over 10 generations. H1R KO mice were originally a gift from Prof. Takeshi Watanabe (Inoue et al., 1996). HDC KO mice were a gift from Prof. Hiroshi Ohtsu (Ohtsu et al., 1996; Ohtsu et al., 2001). Single nucleotide polymorphism analysis was used to analyze that the strains had at least 99.5% identity with C57BL/6J. Experiments were carried out in male and female mice that were 12-18 weeks old. Mice were group-housed and standard food pellets (Scanbur, Sweden) and water were available *ad libitum*. All animals were naïve to drug treatments except for the locomotor activity study where all animals received 3 drug treatments in randomized order. The wash-out period was at least 1 week between each locomotor activation study. The total number of animals used in these studies was 152. Mice were maintained in the Laboratory Animal Centre (LAC) of the University of Helsinki. The principles of the Finnish Act on the Use of Animals for Experimental Purposes were followed in conducting these studies and the protocols were approved by the Animal Experiment Committee of the State Provincial Office of Southern Finland.

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Drug treatments

Apomorphine, haloperidol, MK-801, (-)-quinpirole and (±)-SKF-38393 were from Sigma-Aldrich, St Louis, MO, USA. D-amphetamine (Dexedrine®) was from GlaxoSmithKline, Brentford, UK. Haloperidol was dissolved in 4 % glacial acetic acid (m/m), pH was adjusted to 5.5 with 0.1 N NaOH and the solution was sterile-filtered. All other drugs were dissolved in sterile 0.9 % saline. The drug doses correspond to free bases of the compounds. Injections were given intraperitoneally (i.p.). All treatment groups were formed using randomization.

Acoustic Startle Response

The acoustic startle response and PPI were measured using a two-unit automated startle system (Startle Reflex System, Med Associates Inc., St. Albans, VT, USA) as described earlier (Leppa et al., 2011). The first set of H3R KO and their control mice were tested with the Startle Reflex System version 4.01 and all the rest of the mice (both KO and control animals) were tested with the newer Startle Reflex System MED-ASR-PRO1 (Med Associates Inc.). Both systems were calibrated. Mouse was placed in a plastic cylinder that was then mounted in an illuminated and sound-attenuated chamber above a piezoelectric sensor. Movements of the animal in the cylinder were detected by a piezoelectric sensor, digitized and analyzed by Startle Reflex System software (4.01/SOF-825, Med Associates Inc.). The sensitivities of the two chambers were calibrated and adjusted to be identical. Background noise of 62 dB (4.01/ASP-PRO1) and acoustic stimuli were delivered through speakers in the ceiling of the chambers. Mice were acclimated to the cylinders before the experiments. Acoustic startle responses to stimuli of different intensities were determined. The test session began with a 5-min acclimation, followed by three blocks of trials containing stimuli (40 ms) of different intensities (70-120 dB) in a pseudorandom order. Background noise was on during the acclimation period and throughout the sessions. The startle

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amplitude was defined as the peak amplitude that occurred during the first 100 ms after the onset of the startle stimulus.

Prepulse inhibition (PPI)

PPI was tested with a minimum of three days after startle testing. For habituation, the test session began with six trials of 95 dB stimuli (40 ms). This was followed by two blocks containing startle stimuli (95 dB) alone or combined to a prepulse stimulus (20 ms). The prepulse stimulus was also delivered alone five times during the session. Two to four different prepulse intensities (70-76 dB) were used in order to get a comprehensive presentation of the PPI. The order of different type of stimuli was pseudorandom. The effects of D2R antagonist haloperidol (0.75 mg/kg), apomorphine (1.0 and 5.0 mg/kg) and NMDA antagonist MK-801 (1.0 mg/kg) on PPI were tested in H3R KO mice. Drugs were administered 30 min prior to PPI experiment. PPI in percentage was calculated from the formula: %PPI = [(amplitude of startle pulse alone – amplitude of startle pulse when preceded by a prepulse)/amplitude of startle pulse alone] ×100.

Locomotor activity

After habituation (60-90 min) in the locomotor activity chamber, animals were injected with saline, SKF-38393 (10 mg/kg), quinpirole (0.5 mg/kg) or d-amphetamine (5 mg/kg). The activity of the mice was recorded by Ethovision® Color-Pro 3.0 video-tracking software (Noldus Information Technology, Wageningen, the Netherlands). Distance moved (cm) and velocity (cm/min) were analyzed from the recorded data.

Semi-quantitative Western blotting

Mice were injected with saline, SKF-38393 (10 mg/kg) or quinpirole (0.5 mg/kg) and decapitated 20 min after drug injections. Prefrontal cortices (1.5-3.5 mm from Bregma) and striata (0.5-1.5 mm

from Bregma) were dissected on ice and immediately freezed on dry ice. Samples were kept at -80°C until analyzed. Samples were weighted and homogenized by sonication on ice in lysis buffer (50 mM Tris [pH 7.4], 5 mM EDTA, 1 mM EGTA, 0.1 % Triton-X-100, 5 mM sodium fluoride, 1 x HALT™ phosphatase inhibitor cocktail [Thermo Fisher Scientific, Rockford, IL, USA], Mini Complete protease inhibitor [Roche Diagnostics, Mannheim, Germany]). Homogenates were centrifuged at 13 000 x g (5 min, +4°C) and the protein concentration of the supernatant determined by Bio-Rad Protein assay (Bio-Rad Laboratories, Hercules, CA, USA). Samples (40-50 µg of total protein/well) were separated based on protein size by 10 % SDS-PAGE gel and transferred to a nitrocellulose membrane with eBlot® protein transfer system (Genscript, Piscataway, NJ, USA). Phosphorylated forms of ERK1/2 and AKT were detected with the mouse anti-phospho-ERK1/2-44/42 (#9106) and rabbit anti-phospho-AKT (#9271) primary antibodies. The unphosphorylated forms of the corresponding proteins were detected with rabbit anti-ERK1/2-44/42 (#9102) and rabbit anti-AKT (#9272) primary antibodies. All antibodies were manufactured by Cell Signaling and used at dilutions 1:1000. The following infrared-labeled secondary antibodies (IRDye®, LI-COR Biosciences) were used at dilutions 1:10 000-1:30 000: 800cw Goat anti-mouse IgG1 for monoclonal primary antibodies and 690 LT Goat anti-rabbit for polyclonal primary antibodies. The signal was detected using Odyssey® Imaging System and quantified with the LI-COR Image Studio software. The values were corrected by dividing the signal intensity of the phosphorylated protein with that of the corresponding unphosphorylated protein.

Radioactive *in situ* hybridization

Selective and specific oligonucleotide probes designed for mouse D1R, D2R, tyrosine hydroxylase (TH) and dopamine transporter (DAT) were used to quantify the expression of the corresponding mRNAs. The length of the probes was 43 bases and the nucleotide sequences were as follows:

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D1R (ATGGACTGCTGCCCTCTCCAAAGCTGAGATGCGCCGGATTTGC), D2R (GCTTTCTTCTCCTTCTGCTGGGAGAGCTTCCTGCGGCTCATCG), TH (GGTAGGTTTGATCTTGGTAGGGCTGCACGGCTGCTGTGTCTGG) and DAT (TCAGCCACGCTACAGTCTGCAGAGCCAACAGGCCTGCATTTCC). The protocol for the hybridization has been described previously (Lintunen et al., 1998; Vanhanen et al., 2013).

Data analysis

Statistical analyses were performed using GraphPad Prism software. Prepulse inhibition, locomotor activation, Western blotting, striatal *in situ* hybridization data were analyzed with 2-way ANOVA followed by Sidak's or Bonferroni posttest. Student's two-tailed t-test was used to analyze velocities of locomotor activation data. Values exceeding more than two standard deviations from the group mean were excluded from the analysis.

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Results

Impaired startle responses in H1R knockout mice

The startle responses in H3R KO and HDC KO mice were similar to their wild type controls (Fig. 1A, C) whereas H1R KO mice displayed significantly lower startle responses as compared with their controls (interaction for genotype and stimulus intensity $F_{1,20}$=5.07, P=0.0357, 2-way RM ANOVA, Fig 1B).

Lack of H3R leads to impaired prepulse inhibition

H3R KO mice displayed lower PPI responses as compared with their wildtype controls indicated by a significant genotype effect with RM 2-way ANOVA (Fig. 2, $F_{1,22}$=8.16, P=0.0092). Sidak's posttest revealed that the 70+95 dB prepulse-pulse combination of H3R KO mice was significantly different from controls (P<0.05). The PPI responses in H1R KO and HDC KO mice were similar between the genotypes (genotype effects respectively $F_{1,20,}$ = 2.25, P=0.1493; $F_{1,18}$=3.74, P=0.0688).

Modulation of prepulse inhibition by drug treatments in histamine H3R KO mice

Treatment with dopamine D2R antagonist haloperidol (0.75 mg/kg) did not significantly alter the PPI in control mice but increased PPI response in H3R KO mice as compared to the baseline response (Fig. 3A) indicated by a significant treatment effect ($F_{4,103}$=7.50, P<0.001) by 2-way ANOVA and Sidak's multiple comparison test (P<0.001). Apomorphine (1.0 or 5.0 mg/kg) did not alter the PPI responses in wildtype mice but increased the PPI in H3R KO mice significantly (P<0.01 and P<0.05 for 1.0 mg/kg and 5.0 mg/kg respectively). NMDA receptor antagonist MK-801 did not significantly alter PPI in wildtype or H3R KO mice. Some of the drug treatments affected the startle responses significantly indicated by a significant treatment effect in 2-way ANOVA ($F_{1,101}$=25.02, P<0.0001). Haloperidol increased startle responses in H3R KO mice

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(P<0.01) and MK-801 increased startle responses in both wildtype (P<0.01) and H3R KO mice (P<0.0001).

Locomotor activity of H3R KO and wildtype mice in response to dopaminergic drugs

There was no difference in the basal locomotor activity of H3R KO and wildtype mice as expected from our previous study (Nuutinen et al., 2011b). Interestingly, amphetamine-induced (5 mg/kg, i.p.) stimulation of locomotor activity was higher in H3R KO mice (Fig. 4A, n=12). A significant genotype x time interaction confirmed these findings ($F_{9,198}$=3.07, P=0.0018). The velocity was also higher in H3R KO mice (Fig. 3B, P=0.0331). In the D2R/D3R agonist quinpirole treated (0.5 mg/kg, i.p.) animals there was also a significant interaction ($F_{17,578}$=2.16, p=0.0045) confirming that both time and genotype affect the result and indicating that the effect of quinpirole is altered in H3R KO mice compared to wildtype controls (Fig. 4C, n=18). The velocity of H3R KO mice was also lower than that of the controls after quinpirole treatment (Fig. 4D, P=0.0007). D1R agonist SKF-38393 (10 mg/kg, i.p.) induced a stimulation that was similar in both H3R KO and wildtype mice confirmed by the lack of interaction (Fig. 4E, $F_{17,578}$=1.38, P=0.1374, n=18). The velocity after SKF-38393 was also unaltered in H3R KO mice (Fig. 4F). Distances moved were analyzed by RM 2-way ANOVA followed by Bonferroni posttest and velocities were analyzed by Student's two-tailed t-test.

Striatal dopaminergic signaling is altered in H3R KO mice

Quinpirole (0.5 mg/kg, i.p.) increased ERK1/2 phosphorylation (phosphorylated at Thr202 and Tyr204) in the striatum (Fig. 5A, P<0.01, n=4-5) but not in the PFC 20 min after drug administration in wildtype but not in H3R KO mice. Quinpirole had no effect on AKT (phosphorylated at Ser473) activation in either one of the genotypes in the studied brain regions (Fig. 5B). SKF-38393 (10 mg/kg, i.p.) induced a modest, but significant phosphorylation of

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ERK1/2 in wildtype but not in H3R KO mouse striatum (Fig. 5C, $P<0.05$, n=4-5). No SKF-38393-induced ERK1/2 phosphorylation was observed in PFC in either wildtype or H3R KO mice. SKF-38393 had no effect on AKT phosphorylation in either one of the genotypes in the studied brain regions (Fig.5D). The data was analyzed by 2-way ANOVA and Bonferroni posttest.

Expression of dopaminergic markers in H3R KO mice

The study revealed an overall difference in the mRNA expression of D1R in the striatum confirmed by a significant genotype effect by 2-way ANOVA (Fig. 6A and B, $P=0.0062$). No differences were found between the H3R KO and wildtype mice in the mRNA expression of D2R in the striatum ($P=0.9967$, 2-way ANOVA) or TH and DAT in the ventral tegmental area (VTA) ($P>0.05$, Student's two-tailed t-test).

Discussion

The major finding of the present study is that lack of H3R leads to impairment of sensorimotor gating accompanied by altered responses to dopaminergic drugs, changes in striatal dopaminergic signaling and lower D1R mRNA expression in the striatum.

H3R KO mice displayed significantly weaker PPI than wildtype controls demonstrating the importance of H3R in sensorimotor gating. A major determinant of acoustic PPI is the hearing threshold (Swerdlow et al., 2008). The startle responses in H3R KO mice were normal suggesting that H3R KO mice do not have loss in hearing. Beyond sensory control, nucleus accumbens and pedunculopontine nucleus (PPTg) are key brain areas responsible for the neural control of baseline PPI (Fendt et al., 2001). Both areas receive glutamatergic innervation and the expression of H3R mRNA within these glutamatergic neurons is dense. Thus, alterations in the neuronal activity of glutamate neurons within nucleus accumbens and pedunculopontine nucleus in H3R KO mice might explain the impaired PPI. We found that the effect of MK-801 was similar in H3R KO and wildtype mice suggesting that at least the glutamatergic NMDA receptors function normally in H3R KO mice. The release of glutamate could, however, be altered in H3R KO mice.

To further examine the mechanism underlying PPI deficiency in H3R KO mice dopaminergic drugs haloperidol and apomorphine were used. Interestingly, we found that both the D2R antagonist haloperidol and nonselective agonist apomorphine increased the PPI to the same level as in controls. Unlike in rats, dopamine receptor agonists increase PPI in mice when administered to nucleus accumbens (Mohr et al., 2007). Thus, similar effect with antagonist and agonist on PPI is not that surprising. In contrast to rats, dopamine receptor agonist-induced effect on PPI in mice is not mediated by D2Rs but by D1Rs (Ralph-Williams et al., 2002; Ralph-Williams et al., 2003). Based on these findings it seems that D1R-mediated control of PPI is altered in H3R KO mice resulting in deficient PPI. In agreement with this, we found that the dopamine D1R mRNA levels were significantly lower in the striatal areas in H3R KO mice as compared with their controls. The

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lower PPI in H3R KO mice could thus be explained by the low level of D1Rs. However, when dopaminergic drugs such as haloperidol and apomorphine are given to H3R KO mice, we hypothesize that the D1Rs are activated more efficiently in the absence of H3Rs (not having their regulating counterpart) and thus result in similar PPI responses as in wildtype mice. Similar locomotor responses to D1R agonist SKF-38393 in H3R KO and wildtype mice could mean that alterations in D1R in H3R KO mice occur in areas responsible for the control of PPI but not for locomotion.

In addition to the deficient PPI, the H3R KO mice were found to be hyper-responsive to amphetamine-induced locomotor activation indicating a schizophrenia-like phenotype. However, this conclusion warrants caution as measurement of PPI is not a diagnostic tool of schizophrenia (Swerdlow et al., 2008). Impairments of PPI are also found in several other neuropsychiatric disorders such as GTS, obsessive compulsive disorder and in autism spectrum disorder. Previous studies have revealed a link between the brain histaminergic system and GTS originating from a mutation found in a family of GTS patients (Ercan-Sencicek et al., 2010). Further, Castellan Baldan et al. (2014) found enhanced startle responses and impaired PPI in HDC KO mice. In contrast, in our study the startle responses tended to be lower in HDC KO mice but the difference was not significant. In agreement with Castellan Baldan and colleagues, PPI tended to be lower, although not statistically, in HDC KO mice. Together, these findings confirm that brain histaminergic system has a role in sensorimotor gating. However, our findings suggest that lack of H3R has more severe consequences on sensorimotor gating that the lack of histamine. Our current findings of alterations in the brain dopamine system in the H3R KO mice confirm that H3R receptor rather than histamine itself is an important modulator of dopaminergic signaling. This is plausible since the H3R is known to be constitutively active and it does not need histamine to be activated (Morisset et al., 2000; Wieland et al., 2001).

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More studies are still needed to fully understand the role of H3R in PPI since there is no consensus on whether H3R antagonists affect PPI or not. Many groups showed initially that the H3R antagonists improve impaired PPI. Irdabisant, BF2.649 and ABT-239 improved PPI in mice (Fox et al., 2005; Ligneau et al., 2007; Raddatz et al., 2012). Later, ciproxifan and BF2.639 failed to reverse the impairment of PPI induced by MK-801 or phencyclidine in mice (Burban et al., 2010). Further, BF2.649, ABT-239 and ciproxifan failed to reverse the disruption of PPI by apomorphine in rats. One study found enhanced PPI following administration of both thioperamide and ciproxifan in DBA/2 mice, whereas in C57BL/6 mice they failed to do that (Browman et al., 2004).. Thus, the role of H3R in PPI seems to be dependent on both the mouse strain and the use on imidazole-based H3R antagonist which affect liver CYP450 metabolism (Brabant et al., 2009). Thus, when administered in combination with drugs using the same route of metabolism, increased/decreased concentrations of the drugs in the blood can be detected. This in turn could lead to misinterpretation of the data.

Apart from the increased hyperactivity by amphetamine, H3R KO mice responded differentially to dopamine D2R agonist quinpirole. This suggests that alterations of D2R-mediated responses of locomotion are altered in H3R KO mice. Quinpirole acts on the autoreceptors and inhibits the release of dopamine (Bello et al., 2011). We hypothesize that after the hypolocomotor phase quinpirole is no longer bound to autoreceptors and thus the release of dopamine is increased. Interestingly, this does not have a stimulatory effect on locomotion in H3R KO mice. Thus, this further supports the hypothesis, that the postsynaptic dopaminergic functions are altered in the absence of H3R. To study the mechanisms further, quinpirole and D1R agonist SKF-38393 were selected as these compounds have earlier been shown to activate ERK1/2 in cell cultures and striatal slices (Yan et al., 1999; Cai et al., 2000; Brami-Cherrier et al., 2002; Chen et al., 2004; Wang et al., 2005; Ferrada et al., 2009; Moreno et al., 2011). We found that both quinpirole and SKF-38393 induced ERK1/2 phosphorylation in wildtype but not in H3R KO mouse striatum. Striatal

postsynaptic H3Rs located on GABAergic medium spiny neurons (MSN) are co-expressed with D1Rs or D2RS (Pillot et al., 2002). H3R forms functional heterodimers with D1Rs and D2Rs and modulates dopaminergic signaling via this receptor-receptor interaction (Ferrada et al., 2008; Ferrada et al., 2009). Moreno et al. (2011) has demonstrated that D1R-H3R heterodimers exist in the brain and the ERK1/2 phosphorylation by H3R agonist was absent in striatal slices of D1R. Here we show that phosphorylation of ERK1/2 by D1R or D2R agonist is absent in H3R KO mice suggesting that H3R is critical for the dopamine receptor-mediated signaling in the striatum but not PFC and giving further support for the tight interaction of the histaminergic and dopaminergic postsynaptic signaling. However, the effects of H3R on the dopaminergic system seem to be complex.

Unlike the H3R KO and HDC KO mice, the H1R KO mice displayed significantly impaired startle responses as compared to their controls. This could be due to a loss in hearing (Swerdlow et al., 2008). However, similar PPI in H1R KO and control mice suggest that the H1R KO mice do not have deficit in hearing. The lower startle responses in H1R KO mice might arise from the deficits in motor functions as H1R KO mice show impaired locomotor activity (Inoue et al., 1996). The normal PPI responses H1R KO suggests that lack of H1R does not lead to major disturbances in sensorimotor gating. This is in agreement with the study by Dai et al. (2005) where the effect of isolation stress on PPI was studied in H1R KO (Dai et al., 2005). The authors did not directly compare the PPI between H1R KO and wildtype mice but based on the data the H1R KO mice seem to show similar PPI as control mice. However, Roegge et al. (2007) suggest that the effect of antipsychotics on PPI are mediated partially via H1Rs.

In conclusion, the lack of H3R leads to impairment in sensorimotor gating, lower D1R mRNA levels and altered dopaminergic signaling in the striatum. All these findings together with our previous findings in addictive behaviors (Nuutinen et al., 2010; Nuutinen et al., 2011a; Nuutinen et al., 2011b; Vanhanen et al., 2013; Vanhanen et al., 2015) indicate that histaminergic and

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dopaminergic systems are in close connection, mainly via receptor level interaction of H3R with D1R and D2R in the striatum. These findings implicate that targeting H3R could offer treatment options for neuropsychiatric diseases where sensorimotor gating is disturbed and dopaminergic and/or glutamatergic neurotransmission is altered such as GTS, motor function disorders and alcoholism.

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Authorship Contributions

Participated in research design: Kononoff, Nuutinen and Panula

Conducted experiments: Kononoff, Nuutinen and Tuominen

Performed data analysis: Kononoff and Nuutinen

Wrote or contributed to the writing of the manuscript: Kononoff, Nuutinen and Panula

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Footnotes

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Figure legends

Figure 1. Acoustic startle response is impaired in mice lacking histamine H1R but is normal in H3R and HDC KO mice**.** Results are expressed as means + SEM of startle amplitudes after various acoustic stimuli in histamine H3R (A), H1R (B) and HDC KO (C) mice. Each mouse strain was analyzed separately by repeated measures 2-way ANOVA followed by Sidak's posttest. There is a significant genotype effect in H1R KO mice (B) indicating impaired startle responses, P=0.0357. n=9-12/genotype.

Figure 2. Prepulse inhibition is impaired in H3R but not in H1R and HDC KO mice. Results are presented as means +SEM of prepulse inhibition when 70 or 76 dB prepulse was presented prior to higher 95 dB acoustic stimulus. Each mouse strain was analyzed separately with RM 2-way ANOVA followed by Sidak's multiple comparison test. **P<0.01, n= 9-12 per group.

Figure 3. Prepulse inhibition of acoustic startle response in H3R KO mice in response to pharmacological treatments. A) Pretreatment with dopaminergic drugs haloperidol and apomorphine (APO) increased the PPI in H3R KO mice and abolished the difference between the genotypes seen without medication (baseline). MK-801 did not significantly alter PPI in either of the genotypes. Results are shown as mean prepulse inhibition percentage +SEM analyzed by 2-way ANOVA and Sidak's multiple comparison test. *P<0.05 from the wildtype baseline; $^{###}$P<0.001, $^{##}$P<0.01, $^{#}$P<0.05 from the H3R KO mice baseline. B) Startle amplitudes following drug treatments at 95 dB stimulus. Results are shown as mean startle amplitude +SEM and analyzed with 2-way ANOVA and Sidak's posttest. **P<0.01 from wildtype baseline; $^{##}$P<0.01 from the H3R KO baseline.

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Figure 4. Amphetamine-induced stimulation of locomotor activity is greater in H3R KO mice as compared to the wildtype controls. Quinpirole but not SKF-38393 induced locomotor response is also altered in H3R KO mice. Mice were habituated for 60-90 min in the test chamber prior to administration of amphetamine (5 mg/kg, i.p.), quinpirole (0.5 mg/kg, i.p.) or SKF-38393 (10 mg/kg, i.p.). A) H3R KO mice are more stimulated by amphetamine than wildtype controls, indicated by a significant time x genotype –interaction. B) The velocity of mice is higher in H3R KO mice after amphetamine treatment. B) In the quinpirole-treated animals there is a significant time x genotype -interaction indicating that the effect of quinpirole is altered in H3R KO mice compared to the wildtype controls. D) Quinpirole-treated H3R KO mice move significantly slower than the wildtype controls. E) SKF-38393-induced a stimulation that is similar in both H3R KO and wildtype mice. F) The velocity after SKF-38393 is unaltered in H3R KO mice. Drugs were administered at time point 0 min. Results are expressed as distance moved within 10 min intervals with means ±SEM. n=12-18/genotype 2-way RM ANOVA followed by Bonferroni posttest. Velocities were analyzed by Student's two-tailed t-test, *P=0.0331, ***P=0.0007.

Figure 5. Lack of striatal ERK1/2 activation in H3R KO mice in response to systemic administration of D2R agonist quinpirole and D1R agonist SKF-38393. Western blotting was used to assess the signal transduction responses to dopaminergic compounds in H3R KO and wildtype mice. A) ERK1/2 is phosphorylated in wildtype mice striatum 20 min after quinpirole (0.5 mg/kg, i.p.) treatment, but in the H3R KO mice the treatment had no effect. In PFC ERK1/2 is not phosphorylated by quinpirole in neither of the genotypes. B) Quinpirole does not induce AKT phosphorylation in the striatum or PFC neither in wildtype nor H3R KO mice. C) ERK1/2 is phosphorylated in wildtype mice striatum 20 min after SKF-38393 (10 mg/kg, i.p.) treatment, but in the H3R KO mice the treatment had no effect. In PFC ERK1/2 is not phosphorylated by SKF-38393 in neither of the genotypes. D) SKF-38393 does not induce AKT phosphorylation in the striatum or

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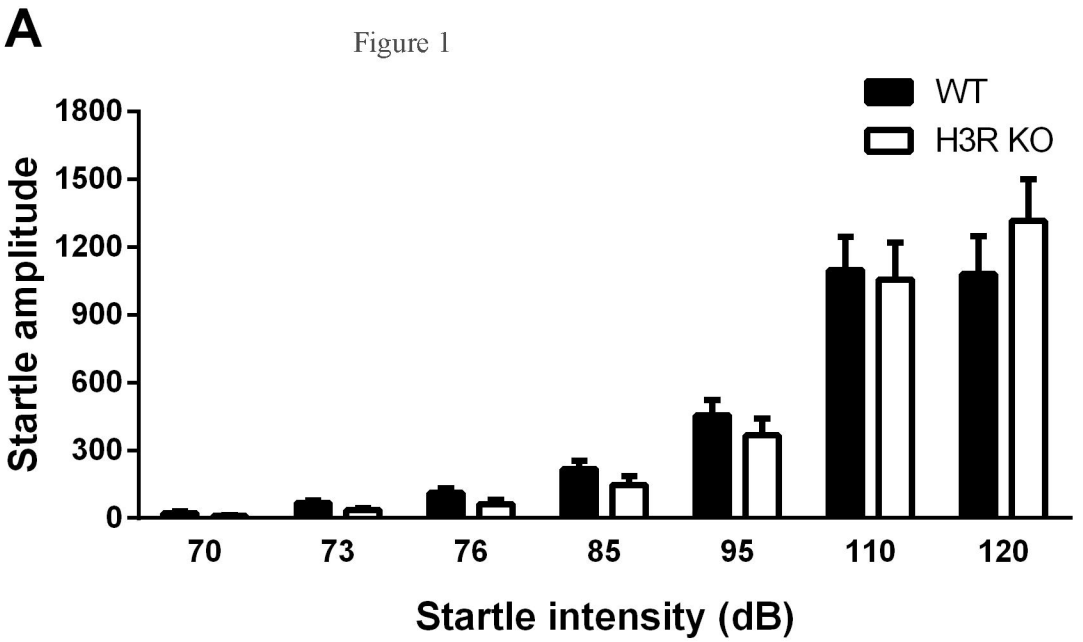
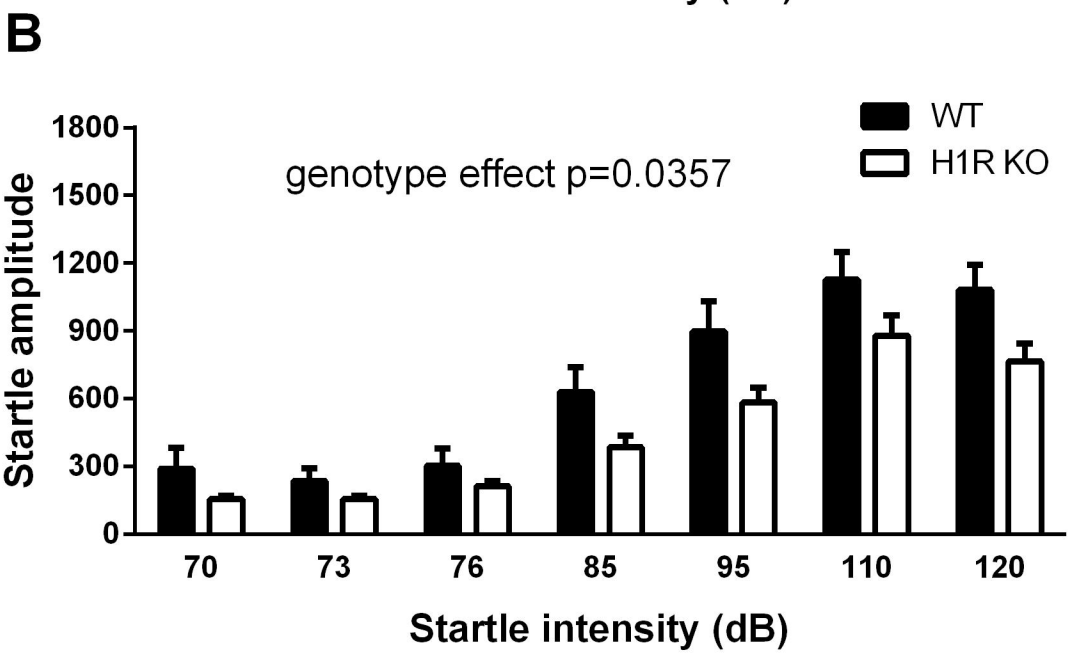
PFC neither in wildtype nor H3R KO mice. n=4-5/genotype, 2-way ANOVA followed by Bonferroni posttest, ** P<0.01, * P<0.05.

Figure 6. The mRNA expression of dopaminergic markers in H3R KO mice. A) Illustration on the right represents the striatal subdivisions that were analyzed from the striatal sections: DL CPu (1); dorsolateral caudate putamen, DM CPu (2); dorsomedial caudate putamen, CPu (3); ventral caudate putamen, AcbC (4); nucleus accumbens core, AcbSh (5); nucleus accumbens shell. Demonstrative figures following radioactive *in situ* hybridization are shown on the left. B) The mRNA expression levels (µCi/mg, average ± SEM) of D1R and D2R in the striatal subdivisions and TH and DAT in the VTA of H3R KO and wildtype mice. The study revealed an overall difference in the mRNA expression of D1R in the striatum (significant genotype effect, P=0.0062, 2-way ANOVA). There is no difference between the H3R KO and wildtype mice in the mRNA expression of D2R in the striatum (P=0.9967, 2-way ANOVA) or TH and DAT in the VTA (P>0.05, Student's two-tailed t-test). n=6-7/genotype.

Figure 1





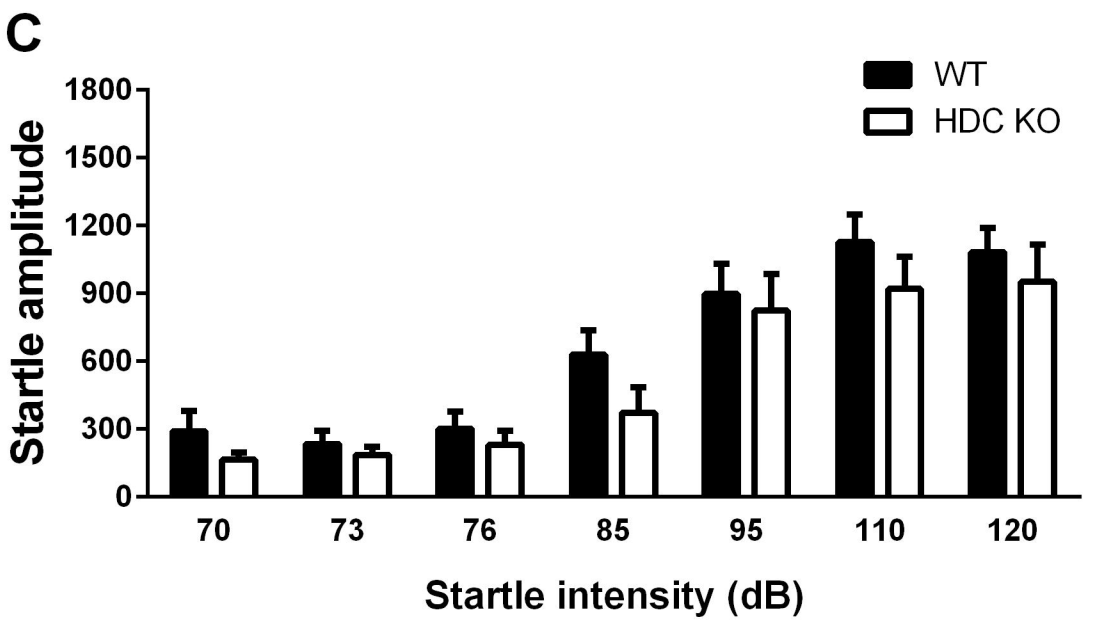
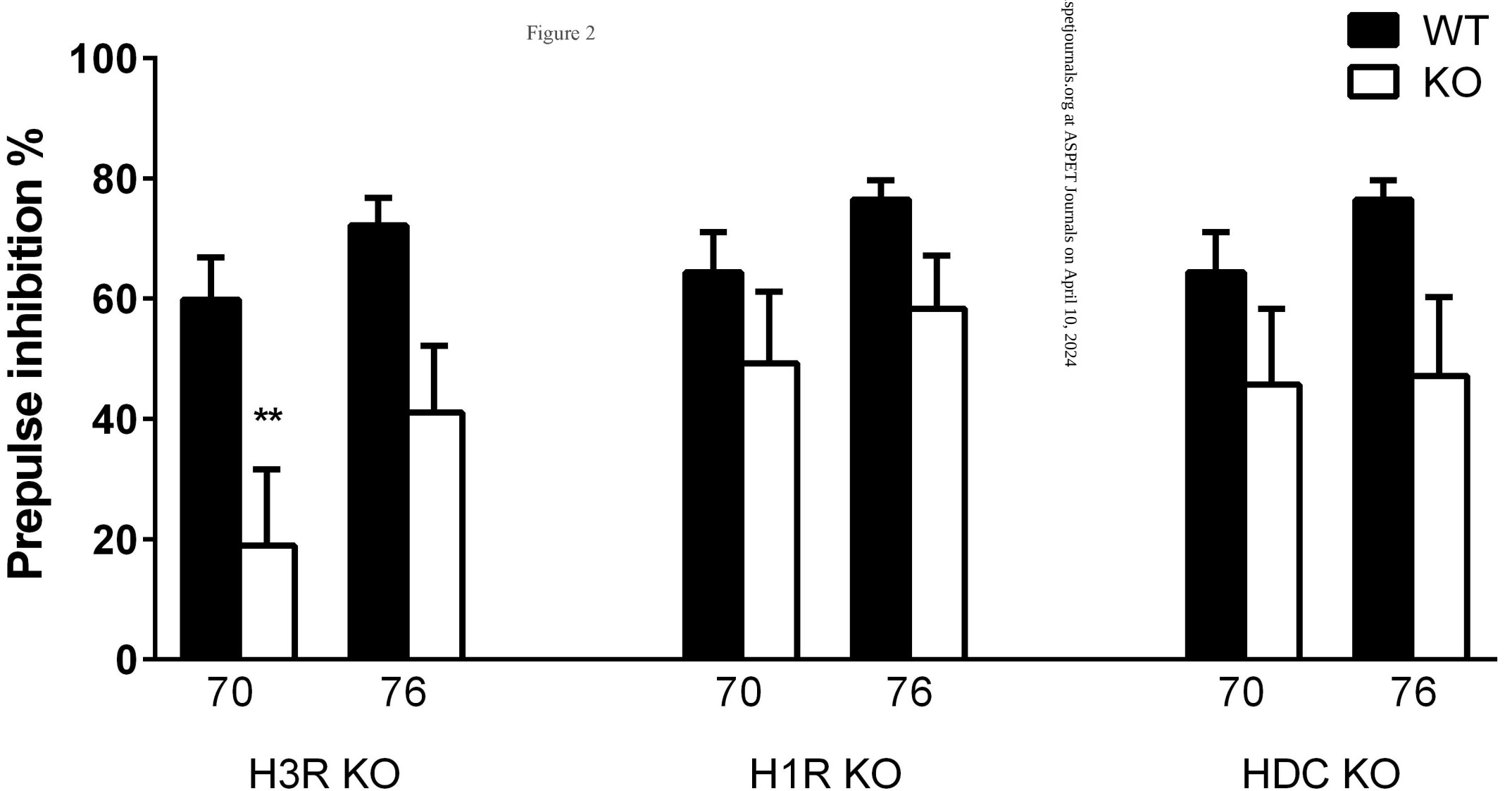


Figure 2



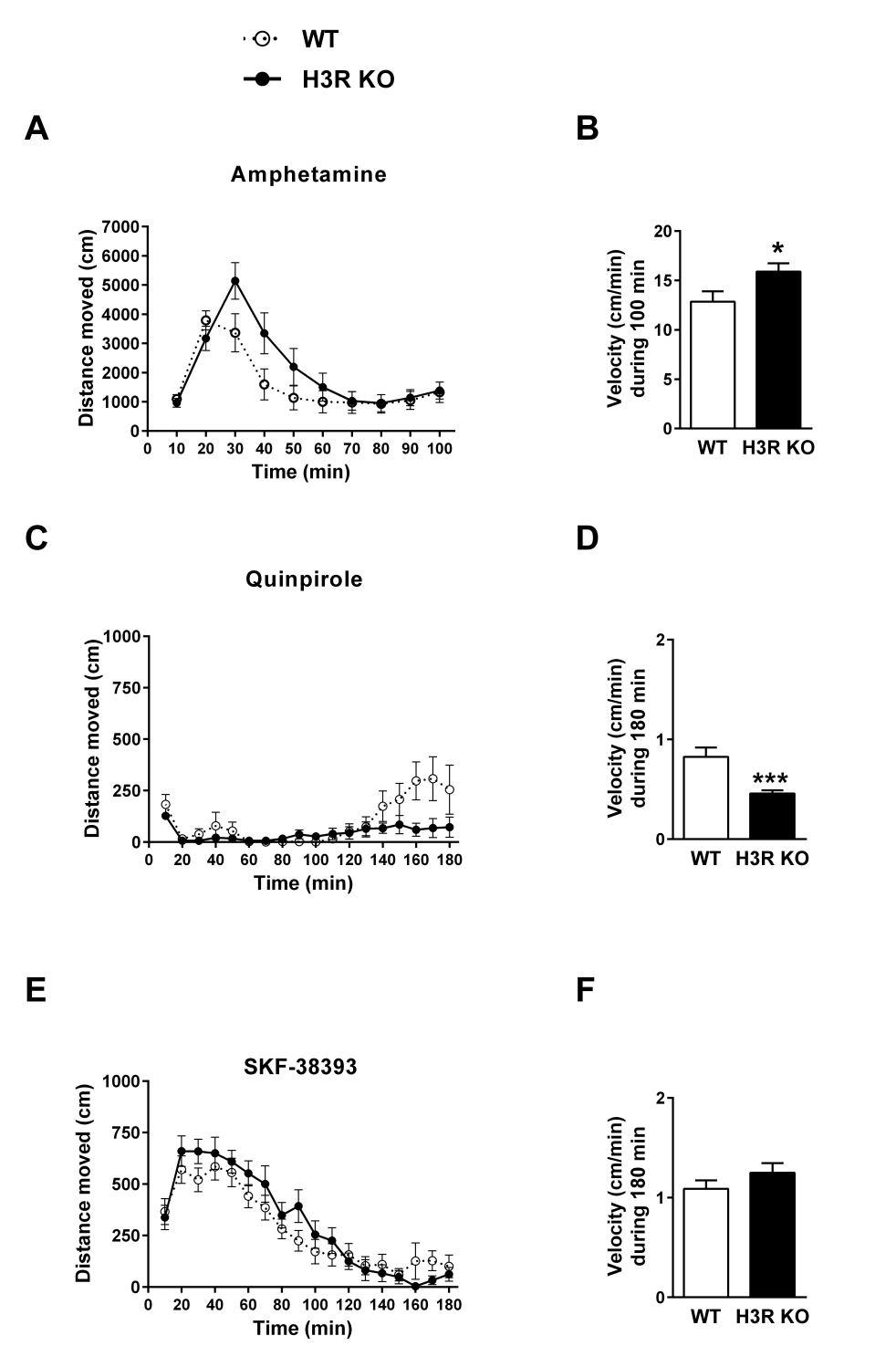

WT
H3R KO
A
Amphetamine
Distance moved (cm)
Time (min)
B
Velocity (cm/min) during 100 min
*
WT
H3R KO
C
Quinpirole
Distance moved (cm)
Time (min)
D
Velocity (cm/min) during 180 min
***
WT
H3R KO
E
SKF-38393
Distance moved (cm)
Time (min)
F
Velocity (cm/min) during 180 min
WT
H3R KO

Figure 4

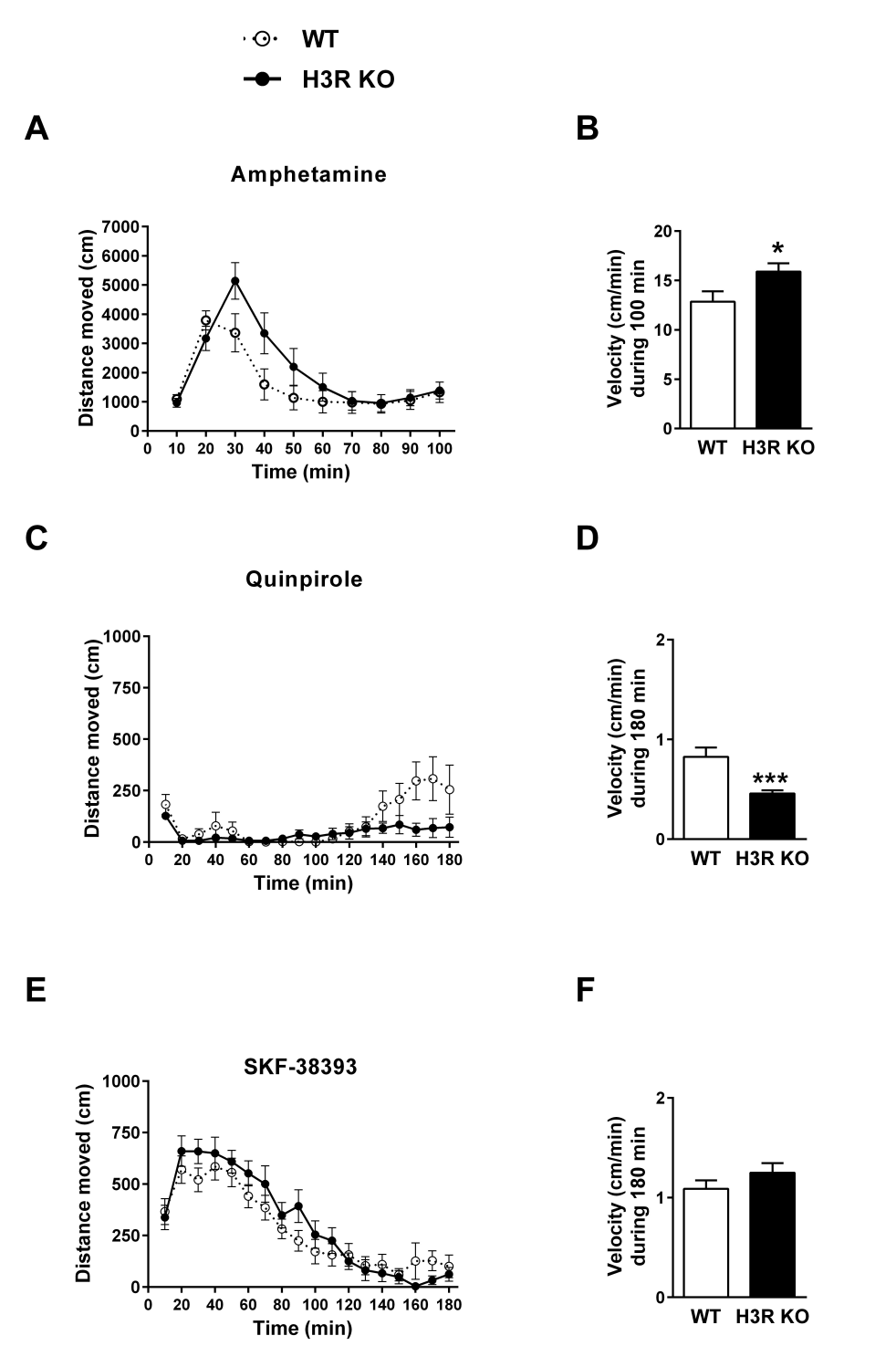
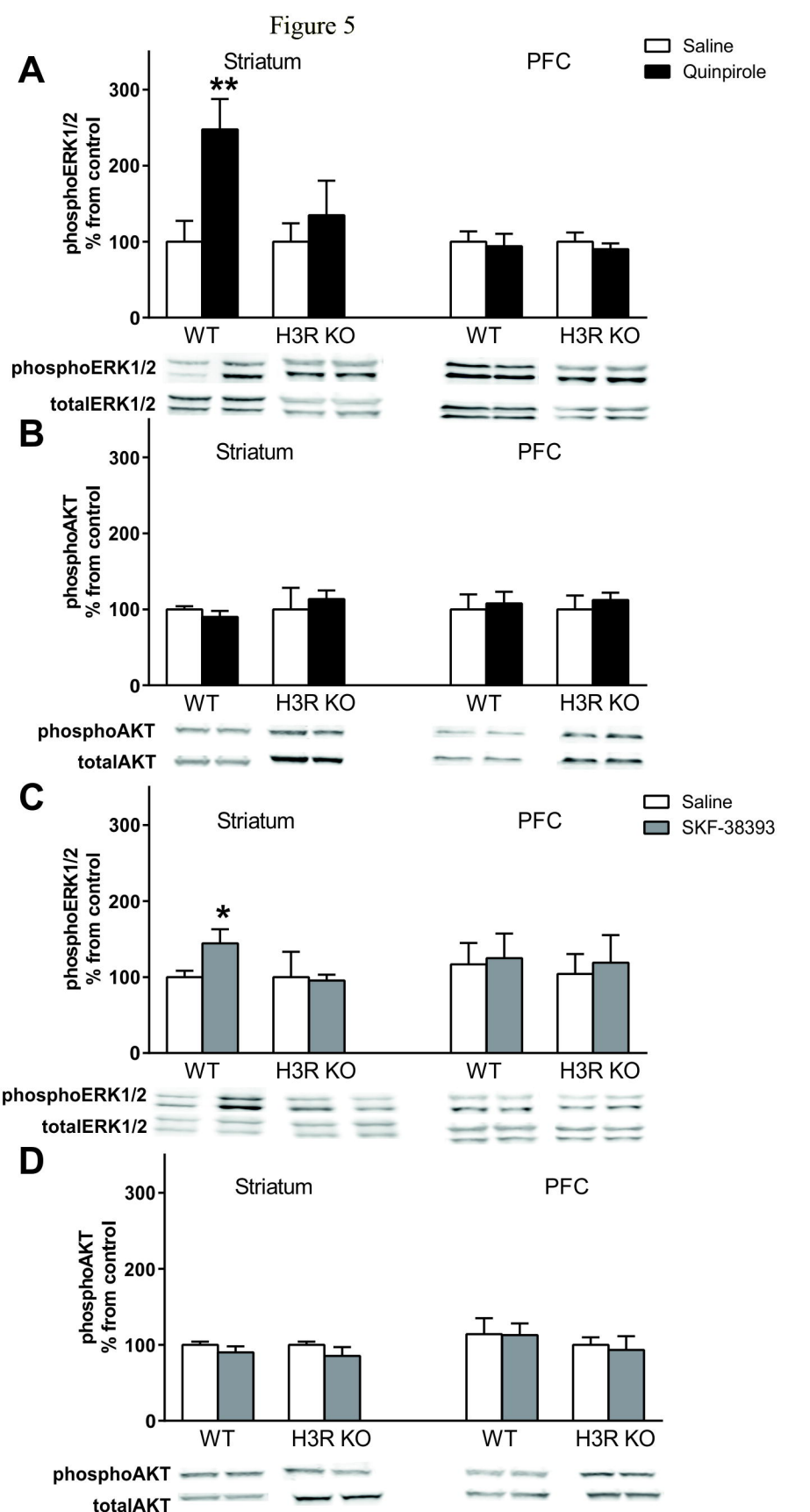

WT
H3R KO
A
Amphetamine
Distance moved (cm)
Time (min)
B
Velocity (cm/min) during 100 min
*
WT
H3R KO
C
Quinpirole
Distance moved (cm)
Time (min)
D
Velocity (cm/min) during 180 min
***
WT
H3R KO
E
SKF-38393
Distance moved (cm)
Time (min)
F
Velocity (cm/min) during 180 min
WT
H3R KO

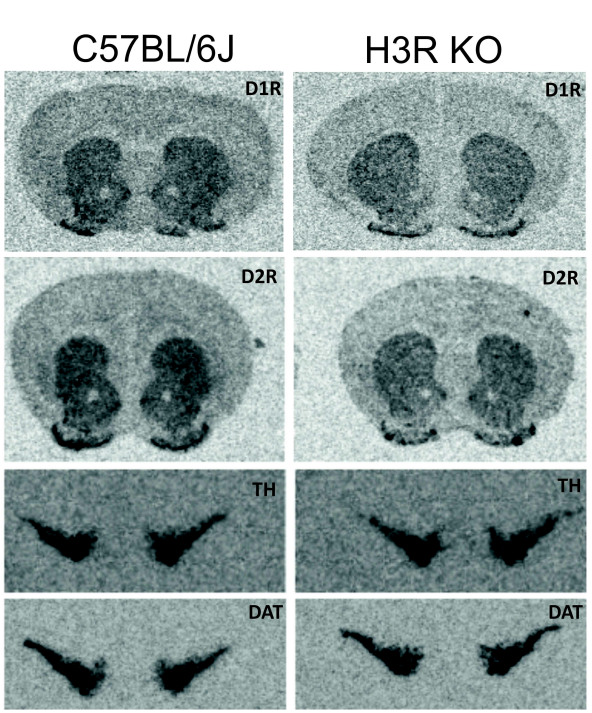
Figure 4

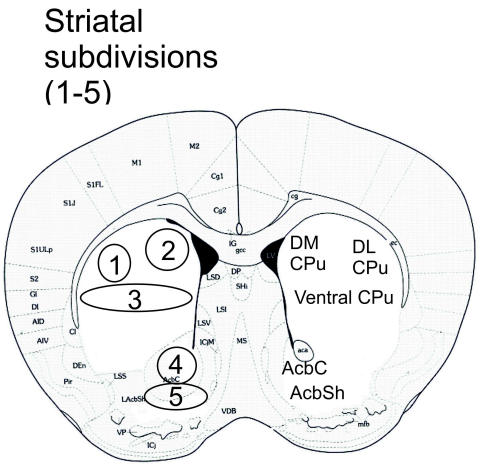
Figure 5


A
Striatum
PFC
Saline
Quinpirole
phosphoERK1/2
% from control
300
200
100
0
**
WT
H3R KO
WT
H3R KO
phosphoERK1/2
totalERK1/2
B
Striatum
PFC
phosphoAKT
% from control
300
200
100
0
WT
H3R KO
WT
H3R KO
phosphoAKT
totalAKT
C
Striatum
PFC
Saline
SKF-38393
phosphoERK1/2
% from control
300
200
100
0
*
WT
H3R KO
WT
H3R KO
phosphoERK1/2
totalERK1/2
D
Striatum
PFC
phosphoAKT
% from control
300
200
100
0
WT
H3R KO
WT
H3R KO
phosphoAKT
totalAKT

A

Figure 6





B

		WT	H3R KO	GENOTYPE EFFECT
D1 receptor	DL CPu (1)	0.35 ± 0.06	0.33 ± 0.06	
	DM CPu (2)	0.32 ± 0.07	0.28 ± 0.07	
	V CPu (3)	0.35 ± 0.06	0.29 ± 0.08	**P=0.0062
	AcbC (4)	0.27 ± 0.06	0.23 ± 0.05	
	AcbSh (5)	0.30 ± 0.05	0.23 ± 0.06	
D2 receptor	DL CPu (1)	0.35 ± 0.02	0.33 ± 0.05	
	DM CPu (2)	0.30 ± 0.02	0.29 ± 0.03	
	V CPu (3)	0.31 ± 0.02	0.30 ± 0.05	P=0.9967
	AcbC (4)	0.25 ± 0.02	0.26 ± 0.05	
	AcbSh (5)	0.23 ± 0.03	0.26 ± 0.05	
TH	VTA	0.38 ± 0.06	0.39 ± 0.03	P=0.4364
DAT	VTA	0.43 ± 0.09	0.45 ± 0.05	P=0.5204