Schizophrenia-like reduced sensorimotor gating in intact inbred and outbred rats is associated with decreased medial prefrontal cortex activity and volume

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Short running title: Brain activity and volume in intact rats differing in PPI

Abstract:

Prepulse inhibition (PPI) of startle response is a measure of sensorimotor gating that is impaired in schizophrenia and in many other clinical conditions. Rat models using pharmacological or surgical strategies reveal that PPI is modulated by the cortico-striatal-pallido-thalamic (CSPT) circuit. Here, we explore whether spontaneous variation in PPI in intact inbred and outbred rats is associated with functional and structural differences in the CSPT circuit. Inbred Roman High- (RHA) and Low-avoidance (RLA) and outbred heterogeneous stock (HS) rats were assessed for PPI, brain activity and volume. Brain activity was assessed by c-Fos expression and brain volume by magnetic resonance imaging. Relevant structures of the CSPT circuit were evaluated, such as the medial prefrontal cortex (mPFC), cingulate cortex, hippocampus (HPC), amygdala, nucleus accumbens (NAc) and dorsal striatum. RHA showed lower PPI than RLA rats, while HS rats were stratified by their PPI levels in three subgroups. Reduced PPI was accompanied by decreased mPFC activity in Roman and HS rats and increased NAc shell activity in HS rats. Low PPI was also associated with decreased mPFC and HPC volumes in Roman and HS rats. This study reports a consistent relationship between decreased function and volume of the mPFC and spontaneous low PPI levels in inbred and outbred intact rats. Moreover, our findings suggest that apart from a hypoactive and smaller mPFC, a hyperactive NAc and smaller HPC may underlie reduced PPI levels. Our results support the notion that sensorimotor gating is modulated by forebrain structures and highlight the importance of the mPFC in its regulation.

1. INTRODUCTION

Prepulse inhibition (PPI) of the startle response is a measure of sensorimotor gating, in which the magnitude of a startle stimulus is attenuated by the presence of a pre-stimulus of lower intensity [1]. PPI impairments are present in several neuropsychiatric disorders [2], including schizophrenia [3,4]. In this sense, studying the neural mechanisms involved in PPI is relevant for progress in our knowledge of the neurobiological basis of schizophrenia-related features [5].

Rodent studies reveal that PPI is modulated by the cortico-striatal-pallido-thalamic (CSPT) circuit involving the prefrontal cortex, thalamus, hippocampus (HPC), amygdala, nucleus accumbens (NAc, via ventral pallidum) and dorsal striatum (via ventral pallidum) efferents to the pedunculopontine nucleus [6–8]. In particular, rat models involving brain alterations have mostly focused on the medial prefrontal cortex (mPFC), HPC and NAc. As shown in these studies, PPI is reduced by either decreased or increased activity in the mPFC [9–12], HPC [5,13–18] and NAc [19–23]. Moreover, several studies using neurodevelopmental models of schizophrenia report PPI deficits paralleled by mPFC and HPC abnormalities, such as models of prenatal administration of phencyclidine [24], prenatal LPS [25] or isolation rearing [26,27]. Additionally, electrical stimulation of the NAc alleviates PPI deficits in poly I:C offspring [28,29], while it disrupts PPI in control-saline offspring. This evidence, derived from rat models involving neural manipulations, is generally consistent with the idea that alterations in the "mPFC-HPC-NAc" circuit [13] play a role in PPI deficits and other cognitive impairments found in schizophrenic patients [30–34].

Here, we explore whether spontaneous variation in PPI in intact inbred and outbred rats is associated with functional and structural differences in the CSPT circuit. Thus, we evaluated neuronal activity (through c-Fos expression) and volume (through structural magnetic resonance imaging –MRI) of relevant regions of the CSPT circuit, such as the mPFC, the cingulate cortex (Cg), HPC, amygdala, NAc and dorsal striatum, in intact rats displaying spontaneous differences in PPI, i.e. the Roman rats and the outbred heterogeneous rat stock.

The inbred Roman high- (RHA) and low-avoidance (RLA) rats were bidirectionally selected for rapid vs. non-acquisition of the two-way active avoidance task, respectively [35,36]. Interestingly, compared with their RLA counterparts, RHA rats show spontaneous reductions in PPI, working memory [37] and latent inhibition [38]. These divergent behavioral profiles, together with

differences in dopaminergic [39,40], serotoninergic and glutamatergic systems [41–43], suggest that RHA rats may be a valid model of schizophrenia-related features. On the other hand, the outbred rat heterogeneous stock (hereafter, HS rats) was developed from a crossing of 8 inbred strains to obtain a stock of rats as heterogeneous as possible [44]. Their genetic heterogeneity makes them a unique tool to study the neurobiological/genetic basis of normal and abnormal complex traits [45,46]. Previous studies suggest that HS rats stratified by low-PPI may constitute a putative model of some schizophrenia-relevant traits [37,47,48].

As reviewed above, PPI is impaired after several alterations that can either increase or decrease activity in the mPFC, HPC and NAc. Thus, we hypothesized that differences in PPI would be paralleled by differences in activity and/or volume within these regions, although the direction of these differences was difficult to predict.

2. MATERIALS AND METHODS

2.1. Subjects

We used male Roman (RHA=28; RLA=28) and HS rats (n=100) from our permanent colony (Dept. Psychiatry and Forensic Medicine, Universitat Autònoma de Barcelona). They were aged 3-4 months, weighing 250-350 g. They were housed in pairs in macrolon cages (50 × 25 x 14 cm) and maintained with food and water ad libitum, maintained under a 12:12 h light-dark cycle (lights on at 08:00 a.m.) and with controlled temperature (22 ±2°C) and humidity (50–70%).

2.2. Experimental procedures

All behavioral and MRI testing was carried out during the light cycle between 09:00-14:00 h. Experiments were performed in accordance with the Spanish legislation on "Protection of Animals Used for Experimental and Other Scientific Purposes" and the European Communities Council Directive (2010/63/EU) on this subject. Every effort was made to minimize any suffering of the animals used in this study.

See experimental overview in Figure 1.

2.3. Prepulse inhibition (PPI)

PPI was conducted in four sound attenuated boxes (SR-Lab Startle Response System, San Diego Instruments, US), as previously described [48]. Briefly, animals were individually located in an acrylic cylinder, which was situated in a dimly illuminated box and on the top of a platform with a sensor that detects the strength made by the rat in each trial. Noise bursts were presented via a mounted 15cm above the cylinder. After 5 min of habituation, 10 "pulse-alone" trials (105 dB(A), SPL, 40ms) were delivered in order to obtain a stable baseline of startle (BL1_Startle). Next, each one of the six types of trials were randomly administered ten times (60 trials in total): (i) Pulse-alone trials (105 dB(A), SPL, 40 ms; BL2_Startle; used to calculate the percentage of PPI); (ii) prepulses of 65/70/75/80 dB(A), SPL (20ms) followed by the pulse stimulus (105 dB(A), SPL, 40ms) with an inter-stimulus interval of 100ms; or (iii) no-stimulus trials (background noise of 55dB). The interval between trials was 15 s (range 10-20 s). The %PPI for each prepulse intensity

was calculated by applying the following formula: %PPI = 100 − (startle amplitude on prepulse trials / startle amplitude on “BL2_Startle” pulse-alone trials x 100).

HS rats were stratified by their average %PPI at pre-pulse intensities 65dB(A) and 70dB(A). Lower pre-pulse intensities, which are closer to the lowest threshold, are known to elicit lower levels of PPI [5]. Therefore, these pre-pulse intensities may be more sensitive to detect differences in PPI. For both experiments with HS rats (studies 3 and 4, see Figure 1), subjects that were one standard deviation below or above the group mean %PPI conformed the Low-PPI and High-PPI groups, while a Medium-PPI group was randomly drawn from the intermediate values.

The “Pulse” and “No-Pulse” control groups for each strain of the Roman and HS rats underwent sessions of pulse-alone trials (i) or non-stimulus trials (iii), respectively, of the same duration as the PPI session.

Assignment of rats to testing box and time of testing was counterbalanced across groups to avoid order effects. Sample size of each experimental group (Figure 1), including n=4-6 animals in control groups, was determined based on previous c-Fos studies suggesting relatively high homogeneity of c-Fos measures in control groups of similar size (e.g. [49,50]).

2.4. Tissue processing, c-Fos immunohistochemistry and microscopy

c-Fos immunostaining was performed with modifications as described earlier [51]. Animals were euthanatized 120min following the PPI, Pulse and No-Pulse sessions. Brains were removed, immersed in isopentane (SIGMA 320404) and stored at 80°C until sectioned. Coronal 20-µm thick sections were cut in cryostat (Leica CM3050S) and thaw-mounted onto slides (Thermo scientific). Slides were stored at -80º. Prior to staining, the slides were allowed to reach RT, then they were immersed in Formaldehyde 3.7-4% for 12 min at RT. After fixation, the slices were washed several times in TBS (0.05M, 0.15M, pH 7.4) and TBS-T (0.05%). Endogenous peroxidase activity was quenched with 3% hydrogen peroxide (SIGMA 216763) in 70% methanol (SIGMA 179337) in 27% TBS, followed by several washes in TBS-T. Afterwards, endogenous protein was blocked incubating the slices with 10% normal horse serum (Sigma, H0146) in “antibody diluent” (TBS-T with 1% (w/v) BSA (SIGMA A9647). Slices were then incubated overnight at 4ºC with the primary

antibody (goat anti-c-Fos IgG, Santa Cruz, SC52G; 1:250). On the next day, slices were washed in TBS-T, and were then incubated with the secondary antibody (biotinylated horse anti-goat IgG, Vector laboratories, BA-9500; 1:200) for 30 min. After washes in TBS-T, the samples were incubated with Peroxidase Streptavidin (HRP conjugated, Jackson immunoresearch, 016-030-084; 1:250) for 30 min. Afterwards, slices were washed in TBS-T, TBS and TB. Then, HRP activity was demonstrated with DAB (50mg DAB (SIGMA, D5637) + 100ml of TB + 33microliters of H_2O_2 (SIGMA 216763)) for 10 min. Afterwards, slices were washed in TB, dehydrated, cleared and cover-slipped. Microphotographs were captured with an Eclipse 80i Nikon microscope attached to a Nikon DXM1200F70 digital camera at 10 magnifications. The regions of study were the mPFC (bregma: from 3.72 to 2.52mm); Cg (bregma: from 3.72 to -1.56mm); dorsal HPC: CA1 of the dorsal HPC (Dorsal CA1), dentate gyrus of the dorsal HPC (Dorsal DG) (bregma: from -2.40 to -3.84mm); ventral HPC: CA1 of the ventral HPC (Ventral CA1), dentate gyrus of the ventral HPC (Ventral DG) (bregma: from -4.80 to -5.52mm); NAc: shell and core (bregma: from 2.28 to 1.56mm); dorsal striatum: caudate-putamen (Striatum CP) and globus pallidus (Striatum GP) (bregma: from -1.80 to -3.12mm); amygdala: basolateral amygdala (BLA) and central amygdala (CeA) (bregma: from -1.92 to -3.36mm). The borders of each area were identified with the help of a rat brain atlas [52]. See further details of the regions of interest in Fig. S1e-k. The ImageJ software ("analyze particles" function) was employed to automatically identify and count the number of c-Fos immunostained nuclei in three histological sections of each brain region/mm^2 and averaged for each animal. Particle size and appropriate grey threshold were set for each region and maintained for all subjects.

2.5. Structural magnetic resonance imaging (MRI)

Settings, parameters and brain region analyses were conducted as previously described [53]. Briefly, we obtained 35 coronal T2-weighted fast spin-echo images from the olfactory bulb to the cerebellum, which allowed us to manually count pixels of the whole brain, mPFC (bregma: from 3.72 to 2.52mm), Cg (bregma: from 3.72 to -1.56mm), NAc (including shell and core sub-regions, bregma: from 2.52 to 0.84mm), HPC (including dorsal and ventral parts, bregma: from -2.40 to -5.52mm), dorsal striatum (bregma: from 2.28 to 0.96mm) and amygdala (bregma: from -1.20 to -5.04mm). The borders of each area were identified with the help of a rat brain atlas [52]. See further details of the regions of interest in Fig. S1a-d. Counting of pixels was performed using

ImageJ software outlined by an experimenter blinded to group status. Volumes of each delimitated area were calculated using the following formula: [(Field of view (3,5×3,5cm^2) / Matrix size (256×256)) x Slice thickness (0.5 mm)] x number of pixels included in delimitated area. The percentage of relative volume (% volume) was obtained by using the following formula: [(volume of a delimitated region)/(total brain volume)] x 100. "% volume" variable was used for analyses in the Roman rat strains, because we found between-strain differences in "total brain volume" measured in mm^3. Sample size of each experimental MRI group from studies 2 and 4 (Figure 1) was determined based on previous MRI studies in rats (e.g. [27,54]).

2.6. Statistics

All the analyses were performed employing the "Statistics Package for Social Sciences" (SPSS). Significance level was set at $p < 0.05$.

A 2x3 ('2 strains X 3 conditions') ANOVA followed by post hoc Duncan's test were used to determine differences between the RHA and RLA rats in the different conditions (i.e. No-Pulse, Pulse, PPI) of the c-Fos study 1.

Student's t-test was used to test significant differences between RHA and RLA rats from study 2.

One-Way ANOVA followed by post hoc Duncan's test were used to determine differences among the HS rats with Low-PPI, Medium-PPI and High-PPI, as well as the control No-Pulse and Pulse groups in studies 3 and 4.

To study associations among variables, multiple linear regression (forward stepwise method) and factorial (direct oblimin; oblique rotation) analyses were applied to c-Fos, MRI and PPI data from studies 1, 3 and 4.

3. RESULTS

3.1. PPI and c-Fos in RHA and RLA rats (study 1):

RHA rats showed lower PPI than their RLA counterparts (Table S1). No differences were found in “BL1_Startle” between the RHA-Pulse and RLA-Pulse groups, while the RLA-PPI group showed higher baseline startle response (both in BL1 and BL2) than the RHA-PPI group (Table S1). However, analyzing groups of RHA and RLA rats previously matched for their baseline startle response, we found that the between-strain differences in %PPI were maintained (Figure S2a,b).

2x3 ANOVA showed a “Strain” effect on c-Fos in the “mPFC” [$F_{(1,30)}$=6.628; p=0.015] and the BLA [$F_{(1,30)}$=7.573; p=0.010; Table 1], as both regions were more activated in RLAs than in their RHA counterparts, and a “Condition” effect in the “mPFC” [$F_{(2,30)}$=4.488; p=0.020], the Cg [$F_{(2,30)}$=3.488; p=0.043; Table 1] and the CeA [$F_{(2,30)}$=4.048; p=0.028; Table 1], revealing that these regions showed higher activation in the PPI and Pulse conditions than in the No-Pulse condition in both strains. Importantly, there was a “Strain x Condition” effect in the mPFC [$F_{(2,30)}$=8.243; p=0.001]. RLA-PPI rats showed higher c-Fos expression than the RLA-Pulse, RLA-No-Pulse and all RHA groups (Duncan's post hoc test, p<0.05 (Fig. 2a); representative microphotographs in Fig. 2b). No significant “Strain” (all $Fs_{(1,30)}\leq$4.112; all $ps\geq$0.052), “Condition” ($Fs_{(2,30)}\leq$2.358; all $ps\geq$0.112) or “Strain x Condition” ($Fs_{(2,30)}\leq$1.737; $ps\geq$0.193) effects were found in other brain regions (Table 1).

3.2. PPI and MRI in RHA and RLA rats (study 2):

RHA rats showed lower PPI than their RLA counterparts (Table S2). No differences were found in “BL1_Startle” and “BL2_Startle” between the RHA and RLA strains (Table S2).

ANOVA showed a Strain effect in volume (%) in the mPFC [$t_{(1,18)}$=2.189; p=0.042], HPC [$t_{(1,18)}$=8.886; p=<0.001] and Amygdala [$t_{(1,18)}$=5.370; p=<0.001] (Student’s t-test; Fig. 3a). RHA strain showed lower relative volume of the mPFC, HPC and Amygdala than the RLAs. No statistical differences were found between groups in “% volume” in other brain regions (all $ts\leq_{(1,18)}$1.737; $ps\geq$0.193; Table S3).

3.3. PPI and c-Fos in HS rats (study 3):

The expected differences in PPI were found after stratification in the three rat subgroups, HS Low-PPI < Medium-PPI < High-PPI, while there were no significant differences in "BL1_Startle" and "BL2_Startle" among the three PPI-stratified groups (Table S4). Albeit non-significant, a numerical trend towards reduced baseline startle in Low-PPI rats is observed. For this reason, we reanalyzed the data from the HS Low-PPI and High-PPI rats after matching them for their baseline startle response amplitude and found that the between-group differences in %PPI were maintained (Fig. S2c-f).

ANOVA showed a Group effect in c-Fos expression in the "mPFC" [$F_{(4,30)}$=7.971; p=<0.001] and "NAc shell" [$F_{(4,30)}$=2.896; p=0.039]. The number of c-Fos positive cells in the "mPFC" was higher in the High-PPI rats than in the Medium-PPI and Low-PPI groups (Duncan's post hoc test, p<0.05; Fig. 2c). Moreover, the High-PPI group was the only one that statistically differed from both the No-Pulse and Pulse control groups. Conversely, in the "NAc shell", the number of c-Fos labeled neurons was greater in the Low-PPI group than in the High-PPI group (Duncan's post hoc test, p<0.05; Fig. 2d). In addition, the Low-PPI group was the only group that statistically differed from the control No-Pulse group. No differences were found among groups in other brain regions (all $Fs_{(4,30)}\leq$2.524; all $ps\geq$0.062; Table 2a). Multiple regressions showed that c-Fos expression in the mPFC and the NAc shell predicted PPI performance (R^2= 0.593; p=<0.001; Table S5). In line with that, factor analysis of PPI, startle and c-Fos variables, pooling HS (study 3) and Roman rats (study 1), revealed a 6-factor solution which grouped PPI and mPFC activation (both with positive sign) in the second factor (Table S6).

3.4. MRI and c-Fos in HS rats (study 4)

We found the expected differences in %PPI variables ("PPI 65_70 pre", "PPI total pre" and "PPI 65 post") among the three PPI-stratified HS subgroups: Low-PPI < Medium-PPI < High-PPI. No differences were found in "BL1_Startle pre", "BL2_Startle pre", "BL1_Startle post" and "BL2_Startle post" among the three PPI-stratified groups (Table S7).

ANOVA showed a Group effect in c-Fos expression in the “mPFC” [$F_{(4,31)}$=4.053; p=0.009], “dorsal CA1” [$F_{(4,31)}$=2.797; p=0.043] and “CeA” [$F_{(4,31)}$=3.624; p=0.016]. The Low-PPI group showed lower number of c-Fos positive neurons in the “mPFC” than the Medium-PPI and High-PPI groups (Duncan's post hoc test, p<0.05; Fig. 2e). In the “dorsal CA1”, the High-PPI group also showed higher c-Fos expression than the Low-PPI and control No-Pulse groups (Duncan's post hoc test, p<0.05, Fig. 2f). In the “CeA”, the control Pulse group showed greater activation than all the PPI groups and the control No-Pulse group, while the PPI groups revealed higher c-Fos expression than the control No-Pulse group (Duncan's post hoc test, p<0.05, Table 2b). No differences were found among groups in other brain regions (all $Fs_{(2,25)}\leq$1.117; all $ps\geq$0.343; Table 2b).

Regarding volumetric measures, ANOVA showed a Group effect in the mPFC [$F_{(2,25)}$=4.750; p=0.018] and the HPC [$F_{(2,25)}$=4.734; p=0.018]. The HS Low-PPI group showed lower mPFC and HPC volumes than the Medium-PPI and High-PPI groups (Duncan's post hoc test, p<0.05, Fig. 3b; see representative MRI images in Fig. 3c,d). No group-related statistical differences were found in other brain regions (all $Fs_{(4,31)}\leq$2.552; all $ps\geq$0.059; Table S8A). Multiple regression showed that volumes of the HPC and mPFC predicted PPI levels (R^2= 0.513; p=0.005; Table S8b). In relation to this, factor analysis of study 4 (including PPI, startle, MRI and c-Fos) showed a 4-factor solution, in which the first factor grouped PPI (pre and post-MRI), mPFC activation and mPFC volume with positive sign (Table S9).

4. DISCUSSION

This work was aimed to investigate the neurobiological mechanisms associated with reduced sensorimotor gating in the Roman and in the outbred heterogeneous HS rats. Our results showed that both neuronal activity and volume of the mPFC, as well as HPC volume, were highly and consistently associated with spontaneous differences in PPI in intact rats.

4.1. PPI is related to mPFC activation and mPFC and HPC volumes in Roman and HS rats

Results of c-Fos expression in the Roman rats (study 1) indicated that the RLA rats showed higher activation of the mPFC than the RHAs during the PPI test. Interestingly, the different conditions markedly affected RLA rats, i.e. much higher c-Fos activation in the “PPI” condition than the “No-Pulse” and “Pulse” conditions, while did not significantly influence c-Fos expression in RHA rats. Moreover, c-Fos results from study 3 revealed that the HS High-PPI group showed higher neuronal activity in the mPFC than all the other four groups, thus suggesting specificity of the mPFC-PPI association. Similarly, c-Fos results in HS rats from study 4 showed that the Low-PPI group displayed lower mPFC activation than the High-PPI, Medium-PPI and Pulse groups. The differences in c-Fos expression between both studies using HS rats (Fig. 2c, e) may be due to habituation, since all animals from study 4 were tested twice in the PPI apparatus. Importantly, it is noteworthy that differences in c-Fos activation between the Pulse and No-Pulse control groups and the PPI groups in study 4 may reflect PPI-related mechanism, although it cannot be excluded that these differences reflect divergences in the experimental history between control and PPI groups, including that PPI groups had undergone MRI measurements under anesthesia.

The main conclusion arising from studies 1, 3 and 4 is that there is a strong and consistent positive relationship between activation of the mPFC and PPI response, which is generalizable through different genetic backgrounds (i.e. different rat strains). Remarkably, this is globally supported by regression analysis revealing that activation of the mPFC (together with NAc shell) predicts PPI levels, and by factor analysis (pool of studies 1 and 3, Table S6). In contrast, the Cg, which is a region adjacent to the mPFC and that has been previously related to schizophrenia ([55] but see also [56]), was equally activated by PPI and Pulse conditions in study 1 in both RHA and RLA and there were no differences among HS rats (study 3). This result reinforces the specificity of the association between mPFC activation and PPI. Importantly, our findings agree with previous

studies showing that PPI is: (i) positively associated with mPFC activation in rats selectively bred for low or high PPI [57]; (ii) impaired after several pharmacological and neurodevelopmental treatments in the mPFC [9–11,24,25]; or (iii) improved in the poly I:C rat model treated with electrical stimulation of the mPFC [28,29]. However, in the only previous study using untreated and unselected animals, there was no difference in c-Fos expression between PPI-exposed and Pulse-exposed mice in the mPFC [49]. One possible explanation for the discrepancy with our findings is that in the mice study, there was just a PPI-exposed group, and animals were not stratified by differential PPI. In fact, the average percentage of PPI in that study was around 50%, which roughly corresponds to Low-PPI rats in the present study 3. Moreover, it is not yet clear that the same neural mechanisms are involved in the regulation of PPI in different species (e.g. mice and rats) [13,49].

Conversely, we found no group-related differences in c-Fos expression in the different HPC sub-regions in studies 1 and 3. These results may seem surprising in light of the literature relating HPC function and sensorimotor gating [5,13,32], although there have also been some controversial findings [49,58]. However, in study 4 the Dorsal CA1 showed higher activation in the HS High-PPI group than in the Low-PPI and No-Pulse groups. In this regard, it is assumed that the Dorsal CA1 is critically involved in several types of memory [59]. As animals were tested twice for PPI in study 4, this may suggest that such an increase of c-Fos activation in the Dorsal CA1 of High-PPI rats is due to a memory process (i.e. habituation of PPI).

In contrast to our findings, previous studies show that PPI is also impaired by manipulations that increase activity in the mPFC [12] or the HPC [14–18]. However, recent evidence suggests that the hippocampal and prefrontal modulation of PPI may be strain-dependent as, for instance, prefrontal disinhibition in Sprague-Dawley rats [12] and ventral hippocampal disinhibition in Wistar rats [16] disrupt PPI, but none of these effects are observed in Lister hooded rats [60,61].

Regarding the MRI results, study 2 revealed that RHA rats showed lower “% volume” of the mPFC and the HPC than the RLAs. These findings agree with previous evidence showing decreased volume of both areas and reduced PPI in RHA compared with RLA rats, as well as a significant positive correlation between HPC volume and PPI [53,62]. In keeping with that, in the present study 4, the HS Low-PPI group showed lower mPFC and HPC volumes than the Medium-PPI and

High-PPI groups. Regression analysis supports these findings, as mPFC and HPC volumes positively predict PPI levels (see Table S8b). In this regard, it is known that decreased brain volume may reflect microscopic changes, including synaptogenesis, dendritic arborization, neurites and neuronal and glial genesis, which can in turn influence on behavioral differences [63–65]. For instance, a study using a “double hit” rat model of schizophrenia reports volume reductions in the mPFC accompanied by several neurochemical alterations in cortical inhibitory circuits in the mPFC, such as a reduction in parvalbumin expressing interneurons, in mRNA levels of calbindin and ERbB4, and in expression of PSA-NCAM and GAD67 [56]. To the extent that macroscopic differences in volume of a specific brain region is parallel by microscopic changes that influence on behavioral differences, our MRI results suggest that the mPFC and the HPC may regulate PPI. Moreover, our experimental strategy (study 4) enabled us to study PPI, MRI and c-Fos in the same subjects. Thus, factor analysis of the most relevant parameters (explaining 64.1% variance) revealed a 4-factor solution, with a first factor grouping PPI variables with mPFC volume and activation (see Table S9), which is globally consistent with the results of group comparisons and regression analyses (studies 3-4). Our findings are in line with previous reports showing both impaired PPI and reduced mPFC volume in several neurodevelopmental rat models [10,26,27,54]. In addition, MRI studies in schizophrenic patients have shown significant correlations between PPI and gray matter volume in the dorsolateral prefrontal, middle frontal and orbital/medial prefrontal cortices [34], whereas HPC volume correlated with PPI in healthy subjects [33]. Moreover, patients with first-episode schizophrenia show significant volumetric reductions in the HPC [66].

The present strategy, which considers the spontaneous variation of PPI in intact rats, may be a parallel of the study of brain function/structure and PPI in humans. Nevertheless, the rat model approach has the obvious advantage of enabling application of invasive procedures and enhanced control of variables. For example, our c-Fos results are in line with a previous functional MRI (fMRI) study in humans that demonstrated that patients with schizophrenia showed lower activation of the frontal and parietal cortical regions than healthy subjects during a PPI test [32]. Importantly, however, the higher spatial resolution of c-Fos over fMRI improves detection of very small nuclei and single neurons involved, which is not applicable to humans [67,68].

4.2. NAc and Amygdala activation during PPI in the Roman and HS rats

The HS Low-PPI group showed higher NAc shell activity than both the High-PPI group and the No-Pulse control group (study 3), revealing an opposite relationship with PPI compared to the findings from the mPFC (see also regression analysis in Table S5). A similar, albeit non-significant, trend was observed in study 1 between the Roman strains, as the mean c-Fos value of the PPI-deficient RHA rats was 35.0(±5.4) vs. 24.5(±5.1) of their RLA counterparts (see Table 1). These findings are consistent with evidence of reduced neuronal activity in the mPFC and increased activity in the NAc of rats selectively bred for deficient sensorimotor gating [57]. Nevertheless, it is worth to point out that the relationship between NAc function and PPI is still a matter of controversy. In fact, PPI is impaired after (i) ablative lesion of the NAc shell [19]; (ii) functional inhibition of the NAc core, but not shell [23]; (iii) amphetamine infusion into the NAc core, but not the NAc shell [69]; or (iv) high-frequency electrical stimulation of the NAc shell in control-saline offspring, while somewhat paradoxically it alleviates PPI deficits in the poly I:C offspring [29]. On the other hand, it has been reported that direct stimulation of alfa-1 and beta adrenoceptors in the NAc shell, but not core, disrupts PPI, and that blocking alfa-1 receptors within NAc shell reverses amphetamine-induced PPI impairments [70]. In relation to this, the acute administration of methylphenidate, which stimulates dopamine transmission in the NAc, elicits an increase in c-Fos expression in both the NAc shell and core, but more markedly in the former, in parallel to PPI impairments in mice [71]. In addition, the disruptive effects of PPI by dopamine agonists seem strain-dependent, as Sprague Dawley are more sensitive to PPI-disruptive effects of apomorphine and amphetamine than Lister Hooded rats [5,69]. To sum up, studies investigating NAc function and PPI have provided mixed and even paradoxical results, which indicates the need for further research. Our results, however, would be in line with those findings suggesting that a hyperactive NAc shell is associated with reduced PPI. Nevertheless, in study 4 no trend for a negative association between NAc shell activation and PPI was observed, which is likely because c-Fos expression was measured after repeated PPI exposure. In this context, there is wide evidence that repeated exposure to a novel environment or stress leads to decreased c-Fos response to those stimuli [72]. Therefore, c-Fos results from study 4 may be to some extent influenced by a process of habituation [73].

On the other hand, previous studies suggest that both the amygdala and NAc may interact to modulate PPI [2]. However, we did not find a specific relationship between PPI and activation in the CeA or BLA. Instead, there was (i) an unspecific “pulse” effect in the CeA, as reflected by higher c-Fos activation in the PPI and Pulse conditions than in the No-Pulse condition (studies 1 and 4); and (ii) a “strain” effect in the BLA in study 1, as the BLA was globally more activated in RLAs than in their RHA counterparts. This finding, together with the increased amygdala volume in RLA rats (see Fig. 3a), is consistent with previous results of strain-related differential stress sensitivity and amygdala volume and function [50,53].

4.3. Conclusions

PPI is a translational measure of sensorimotor gating that is impaired in several human diseases, such as schizophrenia, and is used in many rodent animal models to investigate brain mechanisms underlying these diseases. A common approach in rodent studies has been to assess the impact of specific brain manipulations on PPI, while neural correlates of PPI in humans have preferentially been studied using brain imaging. In this regard, our present work might contribute to bridge the gap between rodent and human studies by investigating structural and functional brain correlates in intact inbred and outbred rats with spontaneous differences in PPI. We report a consistent positive association between PPI and mPFC activity and volume. Moreover, our results suggest that, apart from a hypoactive and smaller mPFC, a hyperactive NAc shell and a smaller HPC may underlie reduced PPI levels. Overall, our findings support the notion that sensorimotor gating is modulated by forebrain structures and highlight the importance of the mPFC in its regulation.

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Figures and legends

Figure 1.

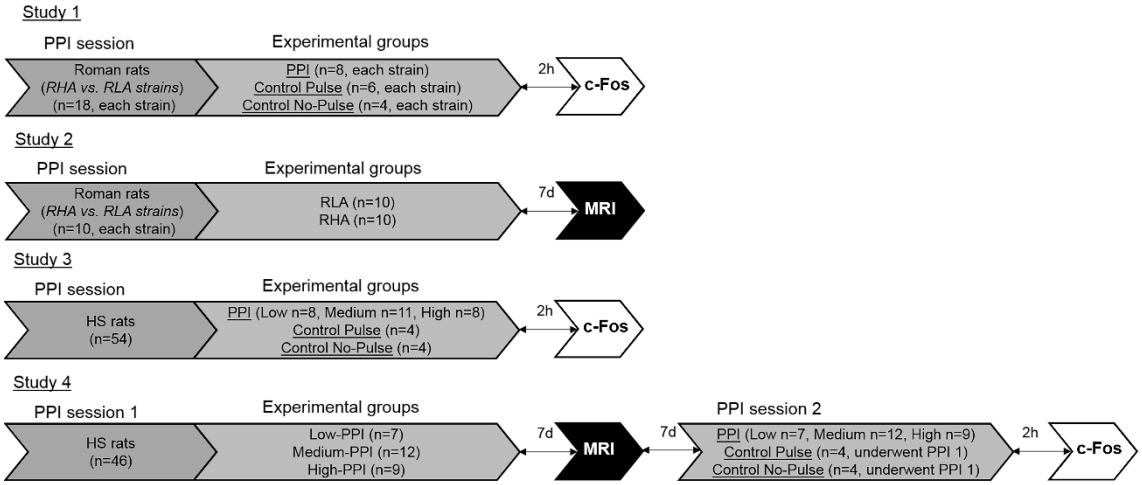

Figure 1. Experimental overview. Study 1: **PPI and c-Fos expression in the RHA and RLA rats**. RHA (n=8) and RLA (n=8; from 6 different litters in both cases) underwent a PPI test. RHA (n=6) and RLA (n=6; from 6 different litters in both cases) underwent a pulse-alone session (Pulse). RHA (n=4) and RLA (n=4; from 4 different litters in both cases) underwent a background noise (No-Pulse) session. They were euthanized 2h later to obtain samples for the c-Fos expression study. **Study 2**: **PPI and MRI in RHA and RLA rats.** RHA (n=10) and RLA (n=10) underwent a PPI test and a single MRI session with a 7-day interval. Rats from both groups were obtained from at least 8 different litters. **Study 3**: **PPI and c-Fos expression in the outbred heterogeneous rat stock (HS rats).** HS rats (n=54, from 40 different litters) underwent a PPI session and they were stratified as Low-PPI (n=8), Medium-PPI (n=11) and High-PPI (n=8) sub-groups by their PPI levels (see "Materials and Methods"). Randomly selected animals underwent control sessions of pulse-alone (Pulse, n=6) or background noise (No-Pulse, n=4). All rats were euthanized 2h after the respective experimental sessions to obtain samples for the c-Fos expression study. **Study 4**: **PPI, MRI and c-Fos expression in HS rats.** HS rats (n=46, from 40 different litters) underwent a PPI session ("PPI session 1") and they were stratified as Low-PPI (n=7), Medium-PPI (n=12), High-PPI (n=9) groups by their PPI levels. Two additional control groups, Pulse (n=6) and No-Pulse (n=4), randomly selected from the whole sample (n=46), also underwent a PPI session ("PPI session 1"). After a 7-day interval, the Low-PPI, Medium-PPI and High-PPI sub-groups underwent a single MRI session, while rats from the Pulse and No-Pulse control groups were transported and kept in the MRI testing room for an equivalent period of time as for the PPI-stratified groups. After another 7-day interval, HS rats from the PPI-stratified groups underwent a second PPI session ("PPI session 2"), while the Pulse and No-Pulse control groups were submitted to a "pulse" or a "background noise" session, respectively. All rats were euthanized 2h after the respective 2nd PPI session ("PPI session 2") to obtain samples for the c-Fos expression study. See variable definitions and further details in "Materials and Methods" section.

Figure 2

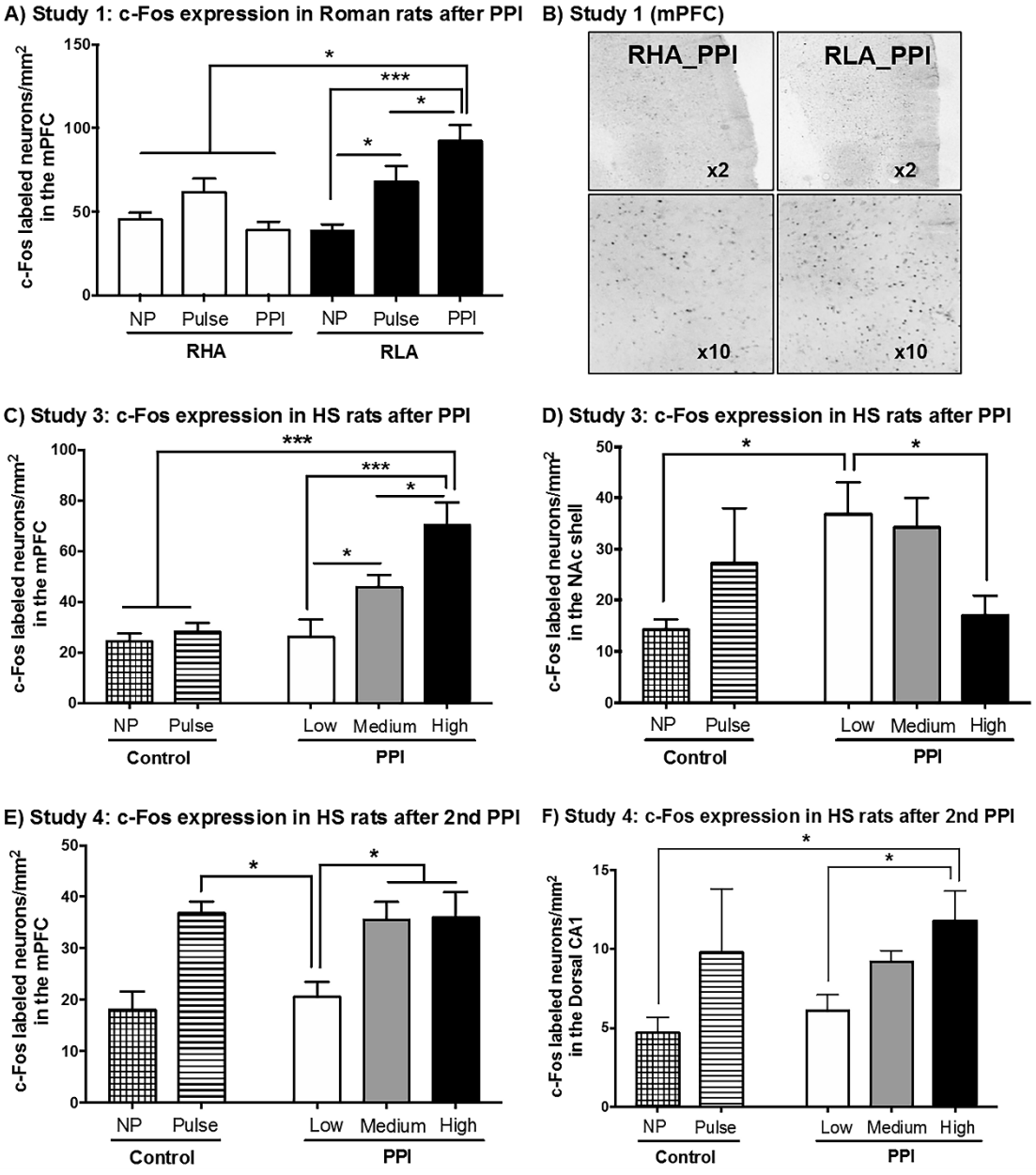
A) Study 1: c-Fos expression in Roman rats after PPI
c-Fos labeled neurons/mm2 in the mPFC
150
100
50
0
*
***
*
*
NP
Pulse
PPI
NP
Pulse
PPI
RHA
RLA
B) Study 1 (mPFC)
RHA_PPI
RLA_PPI
x2
x2
x10
x10
C) Study 3: c-Fos expression in HS rats after PPI
c-Fos labeled neurons/mm2 in the mPFC
100
80
60
40
20
0
***
***
*
*
NP
Pulse
Low
Medium
High
Control
PPI
D) Study 3: c-Fos expression in HS rats after PPI
c-Fos labeled neurons/mm2 in the NAc shell
50
40
30
20
10
0
*
*
NP
Pulse
Low
Medium
High
Control
PPI
E) Study 4: c-Fos expression in HS rats after 2nd PPI
c-Fos labeled neurons/mm2 in the mPFC
50
40
30
20
10
0
*
*
NP
Pulse
Low
Medium
High
Control
PPI
F) Study 4: c-Fos expression in HS rats after 2nd PPI
c-Fos labeled neurons/mm2 in the Dorsal CA1
15
10
5
0
*
*
NP
Pulse
Low
Medium
High
Control
PPI

Figure 2. Impaired PPI is associated with reduced medial prefrontal cortex (mPFC) activation in Roman and HS rats and increased nucleus accumbens (NAc) shell activation in HS rats. a mPFC activation in RLA rats was higher in the PPI condition than in the NP (No-Pulse) and Pulse conditions and was also higher than all conditions in the RHA rats. Conversely, RHA rats did not show differences in mPFC activation among the three conditions. **b** Representative photomicrographs of c-Fos differences in the mPFC (c-Fos expression) between the Roman rats. RHA rats exposed to a PPI session (RHA_PPI) showed lower mPFC activation than their RLA (RLA_PPI) counterparts, as observed both at x2 and x10 magnifications. **c** HS rats stratified for high PPI scores (High-PPI) showed increased neuronal activity in the mPFC compared to HS rats stratified by medium PPI scores (Medium-PPI) and HS rats stratified by low PPI scores (Low-PPI). The Medium-PPI showed higher mPFC activation than the Low-PPI. Additionally, the High-PPI group showed higher mPFC activation than the No-pulse control group (NP, control) and the Pulse control group (Pulse, control). **d** Nucleus accumbens shell (NAc shell) activation in the Low-PPI group was greater than in the High-PPI and NP groups. **e** Following a 2nd PPI session (study 4), the HS Low-PPI group showed lower c-Fos expression in the mPFC than Medium-PPI, High-PPI and Pulse groups. **f** The dorsal CA1 of the hippocampus (Dorsal CA1) in the the High-PPI group showed higher c-Fos expression than the Low-PPI and NP groups. Values are mean±SEM. See "n"/group in Fig. 1. *p < 0.05, **p < 0.01, ***p < 0.001 (Duncan's multiple range test).

Figure 3

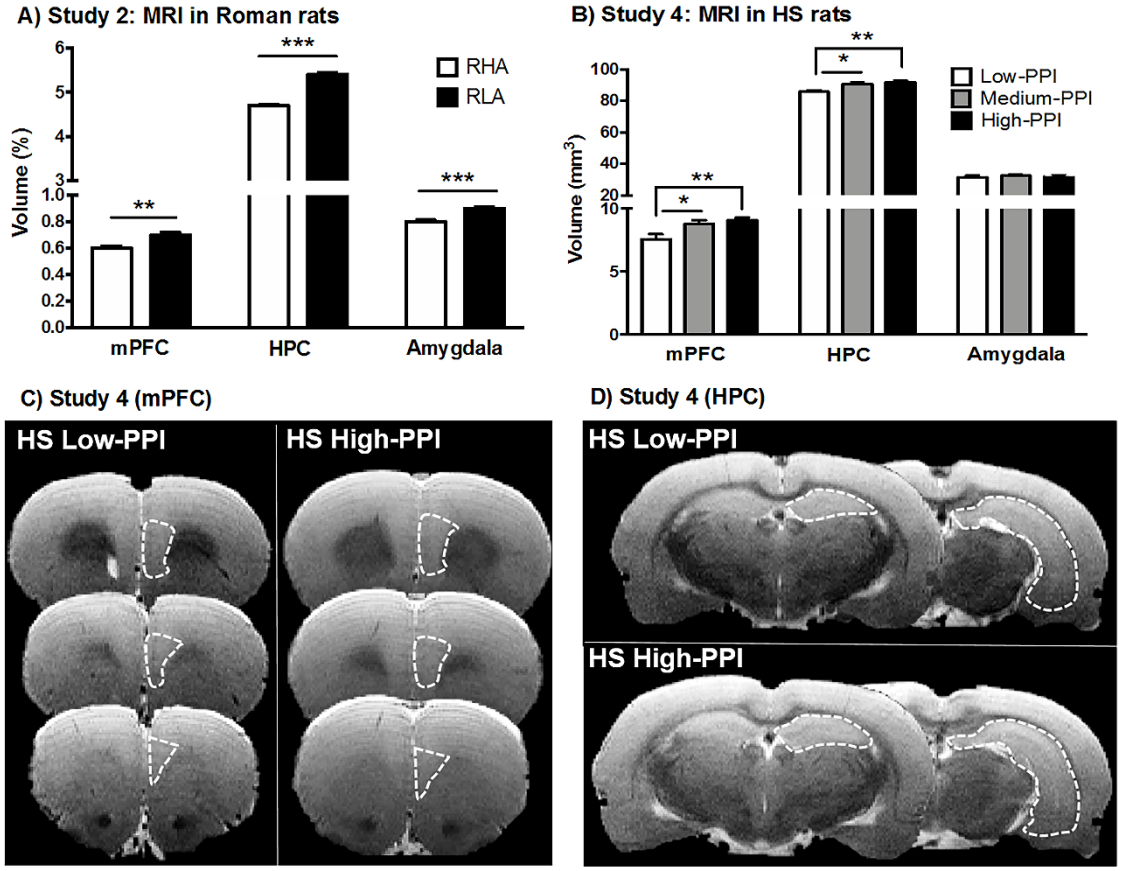
A) Study 2: MRI in Roman rats
Volume (%)
RHA
RLA
mPFC
HPC
Amygdala
B) Study 4: MRI in HS rats
Volume (mm3)
Low-PPI
Medium-PPI
High-PPI
mPFC
HPC
Amygdala
C) Study 4 (mPFC)
HS Low-PPI
HS High-PPI
D) Study 4 (HPC)
HS Low-PPI
HS High-PPI

Figure 3. Lower PPI is associated with reduced MRI volumes of the medial prefrontal cortex (mPFC) and hippocampus (HPC) in Roman and HS rats. a RHA rats, which are impaired in PPI, showed significant reductions in the "% volume" of the mPFC, HPC and Amygdala compared to their RLA counterparts. "% volume" variable was used for analyses in the Roman rat strains, because we found between-strain differences in "total brain volume" measured in mm^3 (see Table S3). **b** The HS rats stratified by low PPI (Low-PPI) showed lower mPFC and HPC volumes than HS rats stratified by medium (Medium-PPI) or high (High-PPI) PPI. No statistical differences were found in the Amygdala. Values are mean±SEM. *p < 0.05, **p < 0.01, ***p < 0.001 (Student's test in a, b; Duncan's multiple range test in c, d). **c-d** Representative coronal MRI images showing volumetric differences in the mPFC (c) and HPC (d) between the HS Low-PPI and High-PPI rats. The HS Low-PPI showed a thinner mPFC and HPC (white dashed lines) than HS High-PPI rats. The borders of each area were identified with the help of a rat brain atlas [52].

Tables and legends

Table 1

Study 1: c-Fos activation in various brain regions of Roman rats after a single PPI session.

	Number of c-Fos labeled neurons/mm²						
	RHA			*RLA*			
	No-Pulse (n=4)	*Pulse (n=6)*	*PPI (n=8)*	*No-Pulse (n=4)*	*Pulse (n=6)*	*PPI (n=8)*	Effects
Cg	31.4 ±1.6	68.6 ±10.5*	61.5 ±11.2*	33.8 ±2.8	52.9 ±9.6*	51.6 ±9.5*	Condition
Dorsal CA1	3.0 ±0.7	3.4 ±1.7	3.0 ±0.6	3.0 ±0.8	4.7 ±0.9	5.9 ±0.9	
Dorsal DG	9.3 ±3.0	8.4 ±1.2	10.1 ±1.3	8.2 ±2.8	16.5 ±2.9	9.0 ±1.3	
Ventral CA1	7.9 ±1.3	11.0 ±1.8	10.6 ±1.6	9.1 ±1.4	8.2 ±1.0	11.3 ±1.1	
Ventral DG	4.2 ±1.7	7.9 ±3.5	11.4 ±2.9	6.8 ±1.7	11.2 ±1.9	7.0 ±1.0	
NAc shell	23.3 ±2.4	28.3 ±5.9	35.0 ±5.4	21.7 ±7.6	38.8 ±7.9	24.5 ±5.1	
NAc core	18.4 ±4.3	21.7 ±4.6	25.7 ±4.6	15.6 ±5.8	17.1 ±4.6	13.5 ±3.0	
Striatum CP	3.7 ±0.7	10.1 ±2.1	7.3 ±1.7	7.5 ±2.4	9.3 ±2.5	11.3 ±1.1	
Striatum GP	3.3 ±1.2	5.4 ±0.8	6.7 ±1.9	4.9 ±3.1	6.4 ±2.0	5.47 ±1.3	
BLA	11.2 ±1.4	15.7 ±2.9	15.0 ±3.2	20.5 ±2.0	20.3 ±2.1	19.2 ±1.9	Strain
CeA	6.5 ±1.7	12.3 ±2.1*	11.1 ±2.1*	1.7 ±2.0	18.5 ±3.9*	15.1 ±1.6*	Condition

Table 1. Study 1: c-Fos activation in various brain regions of Roman rats after a single PPI session. Three groups of RHA and RLA rats were randomly distributed in the background noise control group (No-Pulse), in the pulse-alone control group (Pulse) or the PPI group. Abbreviations: Cg, cingulate cortex; Dorsal CA1, CA1 of the dorsal hippocampus; Dorsal DG, dentate gyrus of the dorsal hippocampus; Ventral CA1, CA1 of the ventral hippocampus; Ventral DG, dentate gyrus of the ventral hippocampus; NAc shell, nucleus accumbens shell; NAc core, nucleus accumbens core; Striatum CP, striatum caudate-putamen; Striatum GP, striatum globus pallidus; BLA, basolateral amygdala; CeA, central amygdala. Effects of "Condition" (with the Pulse and PPI groups showing higher Cg and CeA activation than the NP group) and "Strain" (with RLA rats showing higher BLA activation than RHAs) are indicated in the table (ANOVA). Values are mean±SEM. *$p < 0.05$ vs No-Pulse group of the same strain (Duncan's multiple range test).

Table 2

A) Study 3: c-Fos activation in various brain regions of HS rats after PPI

	Number of c-Fos labeled neurons/mm²				
	No-Pulse (n=4)	*Pulse (n=4)*	*Low-PPI (n=8)*	*Medium-PPI (n=11)*	*High-PPI (n=8)*
Cg	53.2 ±5.4	58.6 ±18.2	66.9 ±9.8	75.2 ±14.3	84.8 ±13.0
Dorsal CA1	4.4 ±0.8	5.1 ±2.7	4.9 ±1.0	5.9 ±1.5	2.3 ±0.7
Dorsal DG	11.4 ±2.3	10.0 ±3.1	11.6 ±2.6	10.2 ±1.0	5.0 ±0.7
Ventral CA1	11.2 ±0.8	11.6 ±2.1	12.2 ±1.6	14.2 ±1.3	12.6 ±2.6
Ventral DG	7.9 ±1.8	19.5 ±7.8	12.8 ±2.0	18.5 ±3.7	17.2 ±3.1
NAc core	5.8 ±2.2	10.2 ±2.8	10.9 ±1.6	17.1 ±3.4	9.7 ±1.7
Striatum CP	9.5 ±3.5	13.7 ±4.5	13.7 ±3.3	12.4 ±2.4	15.6 ±2.5
Striatum GP	5.1 ±1.5	8.1 ±3.7	8.0 ±1.3	6.3 ±1.5	6.3 ±1.2
BLA	10.5 ±4.1	8.4 ±2.6	13.7 ±2.3	19.7 ±2.0	15.8 ±3.0
CeA	7.9 ±1.8	12.3 ±4.3	15.4 ±5.5	10.1 ±1.4	8.0 ±1.5
B) Study 4: c-Fos activation in various brain regions of HS rats after 2nd PPI					
	(n=4)	*(n=4)*	*(n=7)*	*(n=12)*	*(n=9)*
Cg	15.0 ±6.9	12.9 ±.5	18.3 ±2.1	23.1 ±4.2	16.3 ±2.2
Dorsal DG	25.1 ±7.1	22.6 ±2.8	25.4 ±4.0	24.5 ±3.0	24.8 ±3.3
Ventral CA1	9.3 ±1.1	13.0 ±.8	14.9 ±1.3	13.8 ±1.0	15.4 ±2.6
Ventral DG	9.3 ±1.0	8.6 ±2.1	16.0 ±1.7	16.4 ±2.1	12.3 ±1.6
NAc shell	13.5 ±2.3	17.9 ±9.7	21.3 ±5.4	15.0 ±3.0	20.8 ±3.3
NAc core	9.8 ±2.7	11.9 ±4.2	11.2 ±1.8	14.9 ±1.6	12.6 ±1.9
Striatum CP	3.9 ±2.0	13.7 ±4.5	11.8 ±1.3	10.9 ±1.5	12.3 ±2.7
Striatum GP	6.3 ±1.6	8.1 ±1.7	11.6 ±1.8	9.0 ±1.6	10.9 ±1.6
BLA	17.5 ±7.2	26.8 ±3.6	20.7 ±3.4	20.1 ±2.3	23.9 ±3.1
CeA	8.4 ±3.2*	26.8 ±3.2	17.16 ±2.2*	14.67 ±1.6*	18.01 ±3.4*

Table 2. c-Fos activation in various brain regions in HS rats from studies 3 and 4. a In study 3, HS rats were stratified by their PPI scores in rats with low PPI scores (Low-PPI), medium PPI scores (Medium-PPI) and high PPI scores (High-PPI). Moreover, control groups for background noise (No-Pulse) and pulse-alone (Pulse) were used. **b** In study 4, one week after undergoing an MRI session, HS rats were submitted to another PPI session to obtain samples to conduct c-Fos analyses. See further procedural details in "Materials and Methods" and Figure 1. Values are mean±SEM. *, $p < 0.05$ vs. Pulse group in the CeA (Duncan's multiple range test following significant ANOVA effect). Abbreviations as in Table 1.