Prepulse inhibition deficits in inbred and outbred rats and between-strain differences in startle habituation do not depend on startle reactivity levels

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Abstract

The acoustic startle response and prepulse inhibition (PPI) of startle are measures related to information processing, which is impaired in schizophrenia. Some studies have provided inconclusive patterns of association between both measures in rodents. We assessed the influence of baseline startle response on PPI in large samples of Roman high-(RHA) and low-avoidance (RLA) rat strains and in genetically heterogeneous stock (HS) rats. Results show that RHAs exhibit a PPI deficit compared to RLA rats, which is present regardless of the startle response levels. HS rats were stratified in two sub-samples according to their high or low PPI (HS-HighPPI or HS-LowPPI, respectively) scores, and then they were grouped by their differential baseline startle amplitude (high reactivity –HR- or low reactivity –LR-) within each sub-sample. Differences between high- and low-PPI-stratified HS rats remained regardless of their high or low startle amplitude scores. Thus, the impairments in %PPI found in both RHA and HS-LowPPI rats are present irrespective of the relatively high or low levels of startle amplitude in pulse-alone trials. Another objective of the present study was to evaluate whether habituation to the startling stimulus (i.e., pulse) depends on the initial baseline startle response. RLA rats habituated to the startling stimulus more effectively than RHAs regardless of their baseline startle responses. Conversely, there were no differences in startle habituation in the HS rats grouped by their extreme scores of baseline startle. Altogether, these findings suggest a deficit in information processing in RHA rats, which along with evidence indicating that this strain displays other attentional/cognitive impairments, strengthens the validity of the RHA strain as a putative model of schizophrenia-relevant features.

KEYWORDS: schizophrenia, animal model, inbred rats, outbred rats, prepulse inhibition, sensorimotor gating, startle reactivity, startle habituation

Highlights:

RHA rats show a PPI deficit irrespective of the startle reactivity, compared to RLA rats.

RLA rats display higher startle response habituation to pulse-alone trials than RHA rats.

Samples of high/low %PPI HS rats remain different regardless of their startle reactivity.

There are no differences in startle habituation between the HS high- and low-baseline startle subgroups

1. Introduction

Prepulse inhibition (PPI) and the acoustic startle response (ASR) are two measurements of two neurobiological processes that are important to investigate sensorimotor gating and information processing (Swerdlow et al., 2016). PPI is a phenomenon in which a weak stimulus (prepulse) can attenuate the startle response elicited by a subsequent startling stimulus (Swerdlow et al., 2001). ASR is a defensive response to a sudden stimulus that has been extensively studied in many species from zebrafish to humans (Hantsoo et al., 2018). Regarding schizophrenia, many studies have shown that schizophrenia patients display altered arousal patterns to stimuli (Venables, 1966; San-Martin et al., 2020), which can be due to abnormalities in the neuronal circuitry between the brainstem and the midbrain, which regulates the ASR (Koch and Schnitzler, 1997).

The relationship between these two processes in rats has been studied in several papers. According to some authors, baseline startle response and %PPI appear to be dissociable, as suggested by manipulations that change baseline startle but not %PPI (e.g., Feifel et al., 2001; Hoffman and Ison 1980, 1992; Rigdon, 1990; Swerdlow et al. 1986), treatments that alter %PPI but not startle (e.g., Feifel et al., 2001; Furuya et al., 1999; Rigdon 1990; Swerdlow et al., 1990), or interventions that lead to changes of baseline startle and %PPI either in the same or opposite directions (e.g., Acriet al. 1995; al-Amin and Schwarzkopf 1996). According to other studies, although individual startle amplitude and %PPI are not necessarily correlated (Feifel 1999; Feifel et al. 2001; Oliveras et al. 2015; Paylor and Crawley 1997), sometimes they may show low positive associations (Logue et al., 1997; Oliveras et al., 2015; Sanchez-Gonzalez et al., 2016).

In fact, Ison et al. (1997) showed a weak positive association between ASR and the percentage of PPI in CBA/J mice stratified either by high or low baseline startle response. Moreover, recent meta-analytical studies with mice have shown that (i) there is a moderate positive correlation between percentage PPI and baseline startle amplitude (to a 110-dB startling stimulus) in a subgroup of mice showing relatively low ASR amplitudes, (ii) there are no ASR-PPI correlations when considering the intermediate and high ASR subgroups of mice, and (iii) there are negative correlations between ASR amplitude and PPI in these two subgroups when the startling stimulus is 120-dB (Shoji and Miyakawa, 2018). Other studies in mice have also shown that there is a positive relationship between the response elicited by the prepulse and the percentage PPI, thus mice with strong responses to the prepulse also have increased percentage PPI (Csomor et al., 2005). Conversely, other studies have shown a negative correlation between ASR amplitude and PPI in mice and humans (Csomor et al., 2008). Thus, the above studies do not provide a definitive answer to the issue of whether there is an association between ASR and PPI, because they have mostly been carried out in specific mouse strains and because stratifying these animals in subgroups according to their ASR levels the sign of the ASR-PPI association (i.e., correlations) varies depending on the subgroup (Shoji and Miyakawa, 2018). Moreover, similar analyses in humans have not yielded results that can be considered convergent with the above mouse works (Csomor et al., 2008). In their review, Shoji and Miyakawa (2018) point out that there are several factors such as strain, age, sex, species, type (and parametrical aspects) of stimuli, and housing conditions that can induce alterations in both processes (for review see shoji and

Miyakawa (2018), and references therein). Thus, it is important to further explore the impact that the startle response has on PPI scores considering the wide variety of factors that influence PPI levels and the relevance that PPI has as an endophenotype of schizophrenia and other psychiatric disorders (Shoji and Miyakawa, 2018; Swerdlow and Light, 2016).

Startle habituation has also been studied in many species, including Aplysia, C. Elegans, and rats (Rankin et al., 2009). This process can be seen as an indicator of information processing, and it has also been evaluated in schizophrenia patients with contradictory results (Hammer et al., 2011). Thus, even though habituation is not an established biomarker of schizophrenia as PPI, it can give valuable information about the mechanisms that animals use to filter out irrelevant stimuli and focus on new or more relevant ones (Rankin et al., 2009).

Roman high-avoidance (RHA) and low-avoidance (RLA) rats have been psychogenetically selected for good and poor two-way active avoidance acquisition in a shuttle box. Additionally, some studies have focused on the startle response of these strains (Aguilar et al., 2000; López-Aumatell et al., 2009ab; Río-Álamos et al., 2015; Schwegler et al., 1997). In all of these studies, the RLA displayed an increase in the baseline startle response compared to the RHA rats. On the other hand, many experiments have been carried out in our laboratory where RHA rats have consistently exhibited a PPI deficit compared to the RLA rats (Oliveras et al., 2015; del Río et al., 2014; Río-Álamos et al., 2019; Tapias-Espinosa et al., 2018, 2019).

The genetically heterogeneous HS rat stock (i.e., "National Institutes of Health Genetically Heterogeneous Rat Stock") was developed by Hansen and Spuhler(1984)through an eight-way cross from eight inbred rat strains that have been bred for more than 60 generations. The HS rats exhibit levels of PPI and baseline startle that are similar to those of the RLA rats. Moreover, we have used the Roman rat strains and the HS rats to study a wide range of neurobehavioral traits related to schizophrenia (e.g. Esnal et al., 2016; Fernández-Teruel et al., 2006; Giorgi et al., 2019; Oliveras et al., 2015, 2016, 2017; Østerbøg et al., 2020; del Río et al., 2014; Sánchez-González et al., 2016, 2020, 2021; Río-Álamos et al., 2017, 2019; Sampedro-Viana et al., 2021; Tapias-Espinosa et al., 2018,2019).

The main objective of the present work was to assess the influence of the baseline ASR on PPI in large samples of the Roman rat strains and to explore whether the findings are generalizable to HS rats selected for their high and low levels of PPI. We also aimed at evaluating whether there are differences in habituation of the startle response between rats with high levels of PPI (i.e., RLA and high-PPI HS rats) and rats with low levels of PPI (i.e., RHA and low-PPI HS rats).

2. Material and Methods

2.1 Animals

The animals used were naïve (untreated) males of the inbred Roman High- (RHA, n = 164) and Low-Avoidance (RLA, n = 166) rat strains and the genetic heterogeneous rat stock (HS, "National Institutes of Health Genetically Heterogeneous Rat Stock"; n = 348) from the permanent colonies maintained at our laboratory (Medical Psychology Unit, Dept. Psychiatry, and Forensic Medicine, School of Medicine, Autonomous University of Barcelona). These animals were derived from a series of experiments carried out at our laboratory between 2014 and 2018, which involved either naïve (untreated) HS rats, naïve RHA vs. RLA rats, or the comparison among the three stocks (Esnal et al., 2016; Oliveras et al., 2015, 2016; del Río et al., 2014; Río-Álamos et al., 2015, 2019; Sánchez-González et al., 2016; Tapias-Espinosa et al., 2018, 2019).

Rats were approximately 4 months old at the beginning of the experiment (weight range 320–420 g). They were housed in same-sexed pairs in standard (50 × 25 × 14 cm) macrolon cages and maintained under a 12:12 h light-dark cycle (lights on at 08:00 a.m.), with controlled temperature (22 ± 2°C) and humidity (50–70%) and with free access to food and water.

The testing sessions (see procedure below) were performed from 9:00 to 18:00 h in such a way that rats from each strain/stock were counterbalanced with respect to the day and the time of the day of testing. The experiments were approved by the Committee of Ethics of the Autonomous University of Barcelona following the European Communities Council Directive (86/609/EEC) regarding the care and use of animals for experimental procedures.

2.2 Prepulse Inhibition (PPI)

PPI was conducted in four sound attenuated boxes (SR-Lab Startle Response System, San Diego Instruments, US), where the 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 speaker mounted 15 cm above the cylinder. After 5 min of habituation, the session continued as follows (see Figure 1):

- *Baseline-1 (BL1) phase*: 10 "pulse-alone" trials (105 dB (A), SPL, 40 ms; BL1) were delivered to obtain a stable baseline of startle.
- *Baseline-2 (BL2) and "prepulse + pulse" phase*: 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), used to calculate the percentage of PPI); (ii) prepulse of 65/70/75/80dB (A), SPL (20 ms) followed by the pulse stimulus (105dB(A), SPL, 40 ms) with an inter-stimulus interval of 100 ms; or (iii) no-stimulus trials (background noise of 55dB).
- *Baseline-3 (BL3) phase*: Finally, 5 pulse-alone trials (105 dB (A), SPL, 40 ms; BL3) were delivered.

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" pulse-alone trials × 100)].

2.3 Statistical Analyses

Firstly, we evaluated the whole sample of the Roman rats and HS rats in a repeated-measures ANOVA with the levels for the 4 prepulse intensities as a within-subjects factor and the three strains as a between-subjects factor. We also performed a repeated-measures ANOVA with the 3 types of baseline phases (BL1-3, see above) as a within-subjects factor. After that, unpaired Student's t-tests, with Welch correction in case of unequal variances, were applied to investigate the differences among groups.

Then, we performed a repeated-measures ANOVA with the startle response in the 5 trial types (Pulse-alone (BL2), PP65 - 80 + Pulse) as a within-subjects factor and the strains (RHA and RLA) as between-subjects factor. Similarly, repeated-measures ANOVAs were used to evaluate the percentage of PPI in the Roman rats. If there were statistically significant interactions or effects of the groups/strains then we employed an unpaired Student's t-test, with Welch correction in case of unequal variance to elucidate if there were differences among groups. After that, we included the startle reactivity selection (see below) as a between-subjects factor in the repeated-measures ANOVA. Next, we conducted separated repeated-measures ANOVAs for each baseline startle group. Finally, we applied Student's t-tests, with Welch correction in case of unequal variances, to investigate the differences among groups.

For the HS rats, in the first place, we divided them into 2 groups according to their high or low PPI levels. Then we performed two repeated measures ANOVA with the PPI selection as a between-subjects for the startle response in the 5 trial types and the percentage of PPI (within-subjects factors). Then we created the groups according to their startle reactivity in the BL2 phase. After that, we carried out a repeated-measures ANOVA including both PPI and startle reactivity selections. Then we conducted separate repeated-measures ANOVAs for each startle reactivity group. Finally, we applied Student's t-tests, with Welch correction in case of unequal variances, to investigate the differences among groups.

Startle habituation was evaluated through repeated measures ANOVA separately for the Roman rat groups with high or low baseline startle (sample size RHA= 122; RLA=124) with strain and baseline startle selection as between-subjects factors and 5-trial blocks of the BL1-3 phases as a within-subjects factor. Then separate repeated-measures ANOVAs for each baseline startle selection were applied. Finally, we applied Student's t-tests.

For the HS rats (sample size 176) selected for their %PPI levels and their baseline startle (see below), we conducted a repeated-measures ANOVA with both PPI and baseline startle selections as between-subjects factors and 5-trial blocks of the BL1-3 phases as within-subjects factor, and then we conducted separate repeated-measures ANOVAs for each baseline startle group. Finally, we conducted Student's t-test with Welch correction to further explore the significant effects.

In order to create the groups of high and low reactivity of the Roman rats, the sample of each strain was divided into two halves (50^{th}percentile) based on the startle reactivity scores during the BL2 phase (RHA-HR: RHA rats with high reactivity to pulse-alone trials, n = 82; RHA-LR: RHA

rats with low reactivity to pulse-alone trials, n = 82; RLA-HR; RLA rats with high reactivity to pulse-alone trials, n= 83; RLA-LR: RLA rats with low reactivity to pulse-alone trials, n= 83).

Similarly, to generate the HS groups with high and low PPI we divided the sample into two halves (50^{th} percentile) based on total %PPI levels (i.e., %PPI averaged for the 4 prepulse intensities). These two (HS-HighPPI, n =174, HS-LowPPI, n = 174) groups were further stratified (50^{th} percentile) as HR or LR, i.e., high or low reactivity to pulse-alone trials during the BL2 phase, respectively. Thus, HS groups were: HS-highPPI-HR, HS-highPPI-LR, HS-lowPPI-HR, HS-lowPPI-LR (n = 87/group).

Finally, in order to create the groups to study the habituation to the startling stimulus, the Roman rats, and the HS rats with high and low %PPI, were divided into two groups (50^{th} percentile) based on the mean of the first 5 pulse-alone trials of the BL1 phase (RHA-HB: RHA rats with high baseline startle response; RHA-LB: RHA rats with low baseline startle response(n = 61/group); RLA-HB: RLA rats with high baseline startle response; RLA-LB: RLA rats with low baseline startle response(n = 62/group); HS-HB: HS rats with high baseline startle response; HS-LB: HS rats with low baseline startle response(n = 88/group)).

All the analyses were performed with SPSS 17 and a p-value < 0.05 was considered statistically significant for the repeated-measures ANOVA and we adjusted with the Bonferroni correction the two-sided p-value at p< 0.001for the multiple t-test comparisons.

3. Results

3.1 Comparison of RHA, RLA, and HS rats in the main variables of the PPI session

In Table 1 we present the values for the main variables assessed during the PPI session. The overall repeated-measures ANOVA for the four prepulse intensities (within-subjects factor) and the three strains (RHA, RLA, and HS) as between-subjects factor showed a significant "intensity x strain" interaction ($F(4.8, 1620) = 7.80$ $p < 0.001$) as well as the "intensity" factor ($F(2.4, 1620) = 785.97$ $p < 0.001$). Regarding the between-subjects factor we also observed a significant "strain" effect ($F(2, 675) = 63.00$ $p < 0.001$). These results indicated that RLA and HS rats exhibit higher scores than RHA rats through all prepulse intensities (see t-tests in Table 1).

The repeated-measures ANOVA for the three types of pulse-alone trials (BL1-3) revealed a significant "trial-type x strain" interaction and a significant "trial-type" effect ($F(2.9, 606.6) = 5.11$ $p \leq 0.002$, $F(1.5, 606.6) = 224.18$ $p < 0.001$, respectively). The "strain" factor was also significant ($F(2, 419) = 11.73$ $p < 0.001$). In general, it is observed that in the variables that evaluated the startle response in pulse-alone trials the RLA had higher values than the HS rats (see t-tests in Table 1).

3.2 PPI differences between RHA and RLA rats as a function of low- and high- (BL2) reactivity (LR and HR) to pulse-alone stimuli

Repeated-measures ANOVA was carried out with 5 types of trials ("PULSE" alone, PP65+P, PP70+P, PP75+P, and PP80+P) as a within-subject factor and the strain (RHA and RLA) as between-subjects factor (Figure 2A) shows significant "trial-type x strain" ($F(1.5,478.3) = 5.55$, $p \leq 0.001$)and "trial-type" ($F(1.5,478.3) = 359.12$, $p \leq 0.001$) effects. The "strain" effect was not significant ($p \geq 0.123$; Figure 2A). To see whether the RHAs exhibited the expected PPI deficit compared with RLA rats, we run a repeated-measures ANOVA with the percentage of prepulse inhibition (%PPI) for each intensity as a within-subjects factor and the "strain" as a between-subjects factor (Figure 2B). Significant "trial-type x strain" ($F(2.6,865.8) = 7.73$ $p \leq 0.001$), "trial-type" ($F(2.6,865.8) = 441.68$, $p \leq 0.001$), and "strain" ($F(1,328) = 38.28$, $p \leq 0.001$), effects were observed (Figure 2B). The Student's t-tests revealed that the differences in %PPI between the strains in all the prepulse intensities were significant after Bonferroni correction.

Repeated measures ANOVA (5 x 2 x 2) was carried out with 5 types of trials ("PULSE" alone, PP65+P, PP70+P, PP75+P, and PP80+P) as a within-subject factor and the strain (RHA and RLA) and the selection for BL2 startle reactivity (HR vs LR) as between-subjects factors (thus involving RHA-HR, RHA-LR, RLA-HR and RLA-LR groups; Figure 2C, 2E). The results show a significant "trial-type x strain" interaction ($F(1.7,559.3) = 8.36$, $p \leq 0.001$), a significant "trial-type x startle reactivity" ($F(1.7,559.3) = 167.38$, $p \leq 0.001$), as well as a significant effect of the trial-type ($F(1.7,559.3) = 541.11$, $p \leq 0.001$). The "strain" and "startle reactivity" factors were also statistically significant ($F(1,326) = 4.78$, $p \leq 0.029$ and $F(1,326) = 327.90$, $p \leq 0.001$, respectively; Figure 2A). The startle responses were reduced in RLA rats to a greater degree than in the RHA

rats (in PP65+P to PP80+P trials), although no differences were found in the pulse-alone trials (see “PULSE”, Figure 2C, 2E).

To explore whether the RHAs exhibited the expected deficit in %PPI compared with RLA rats and regardless of their BL2 startle reactivity, we run a repeated-measures ANOVA (4 x 2 x 2) with the %PPI for each of the 4 prepulse intensities as a within-subjects factor and the “strain” and “startle reactivity” (BL2) as between-subjects factors (thus involving RHA-HR, RHA-LR, RLA-HR and RLA-LR groups; Figure 2D, 2F). The results revealed a significant “trial-type x strain” interaction ($F(2.7,875.7) = 7.96$, $p \leq 0.001$), a significant “trial-type x startle reactivity” interaction ($F(2.7,875.7) = 10.05$, $p \leq 0.001$) and significant effect of the trial-type ($F(2.7,875.7) = 454.86$, $p \leq 0.001$). The between-subjects factors were also significant, “strain” ($F(1,326) = 40.80$, $p \leq 0.001$) and “startle reactivity” ($F(1,326) = 19.95$, $p \leq 0.001$, Figure 2D, 2F).

To better explore the above “trial-type x startle reactivity” effects and to elucidate the influence of the “startle reactivity” on the PPI measures we conducted separate repeated-measures ANOVA (on “startle amplitude” and on %PPI) for HR and LR groups.

ANOVA on startle amplitude of RHA-HR and RLA-HR rats (Roman rats with high reactivity in the BL2 pulse-alone trials) (Figure 2C) revealed a significant “trial-type x strain” interaction ($F(1.6, 266.5) = 3.75$, $p \leq 0.033$), and a significant “trial-type” ($F(1.6, 266.5) = 361.00$, $p \leq 0.001$). The “strain” effect was not significant ($F(1,163) = 2.37$ $p \leq 0.125$). These results indicate that there are no significant between-strain differences in the overall (whole-session) startle response, but there is a larger reduction of the startle response to prepulse+pulse (i.e., PP65+P to PP80+P) trials in RLA rats, as indicated by the significant interaction. The results of the repeated-measures ANOVA on %PPI for each prepulse intensity (as the within-subjects factor; Figure 2D) revealed a significant “trial-type effect ($F(2.3,377.4) = 244.38$, $p \leq 0.001$) and a significant “strain” effect ($F(1,163) = 11.13$ $p \leq 0.001$) (Figure 2D). We also performed t-tests for each trial type. The results show that regarding the startle response amplitude (Figure 2C) there was no significant difference, and only a tendency to significance appeared in the PP75+P trials (Figure 2C). In the %PPI, there were significant differences between groups in the %PPI75, while in the other prepulse intensities the differences didn't remain significant when the p-value was adjusted for multiple comparisons (Figure 2D).

Repeated-measures ANOVA for the Roman rats that displayed low BL2 startle reactivity (RHA-LR and RLA-LR) was also conducted (Figure 2E, 2F). The results for the startle amplitude in the 5 different trial types revealed a significant “trial-type x strain” interaction ($F(2.5, 409.8) = 12.01$, $p \leq 0.001$) and a significant trial-type effect($F(2.5, 409.8) = 290.90$, $p \leq 0.001$). The “strain” effect was also significant ($F(1,163) = 4.24$ $p \leq 0.041$) (Figure 2E).The results of the repeated-measures ANOVA with the %PPI for each prepulse intensity (Figure 2F) as the within-subjects factor revealed a significant “trial-type x strain” interaction ($F(2.7,447.8) = 6.03$ $p \leq 0.001$), a significant “trial-type”($F(2.7,447.8) = 226.21$ $p \leq 0.001$), and a significant “strain” effect ($F(1,163) = 31.28$, $p \leq 0.001$; Figure 2F). There were differences in startle amplitude in three trial types (PP70+P, PP75+P, PP80+P; Figure 2E), although only the PP70+P remained significant after the p-value was adjusted for multiple t-tests comparisons (Figure 2E). Concerning %PPI, there were significant differences in all the intensities indicating a better PPI performance of the RLA-LR rats (Figure 2F).

3.3 Between-strain differences in habituation to the pulse-alone stimuli, and influence of the initial baseline startle (BL1) response amplitude

In order to explore whether there are differences in the habituation to the pulse-alone stimuli across the session, we conducted a repeated-measures ANOVA with rats selected for their high or low response in the first 5 pulse-alone trials (i.e., during BL1 phase; "baseline startle" factor) of the session (Figure 3). The 50th percentile was the cut-off point, so rats below this percentile were classified as a "low baseline startle" (RHA-LB, n=61 and RLA-LB, n=62), and rats with scores higher than the 50th percentile were classified as "high baseline startle" (RHA-HB, n=61 and RLA-HB, n=62). In the repeated-measures ANOVA, with the "baseline startle" selection (HB vs. LB) and the "strain" (RHA, RLA) as the between-subjects factors, and the pulse-alone trials of the whole session divided into 5 blocks of 5 trials ("trial-block" within-subjects factor; Figure 3), we observed a significant "trial-block" effect ($F(3,723.5) = 145.06$, $p \leq 0.001$), as well as, the interaction between "trial-block x baseline startle"($F(3,723.5) = 89.10$, $p \leq 0.001$), and the "trial-block x strain" ($F(3,723.5) = 13.76$, $p \leq 0.001$). Remarkably, the second-order interaction (trial-block x baseline startle x strain) was also significant ($F(3,723.5) = 6.00$, $p \leq 0.001$). Overall, this second-order interaction and the "trial-block x strain" interaction reflects that RLA rats habituated better to the startling stimulus across the five 5-trial blocks, whereas the degree of habituation across trial blocks was also depending upon the "baseline startle" selection factor (indicated by the "trial-block x baseline startle" interaction). Finally, the "strain" and "baseline startle" effects were also significant ($F(1,242) = 16.26$, $p \leq 0.001$; $F(1,242) = 250.88$, $p \leq 0.001$; respectively), along with the interaction between these two factors ($F(1,242) = 9.87$, $p \leq 0.001$) (Figure 3).

To more clearly elucidate the sources of the above second-order interaction, we conducted repeated-measures ANOVA for the high (HR) and low (LR) baseline startle groups separately. The analysis for the HB groups yielded a significant "trial-block x strain" interaction (Figure 3A), as well as a significant "trial block" ($F(3,359.2) = 10.27$, $p \leq 0.001$ and $F(3,359.2) = 128.93$, $p \leq 0.001$, respectively). The results also show a significant effect of the strain $(1, 121) = 16.20$, $p \leq 0.001$; Figure 3A). The results of the rats with low baseline startle (LB) revealed a significant "trial-block x strain" interaction ($F (3.2, 387.7) = 6.71$, $p \leq 0.001$), and a significant "trial-block" ($F(3.2, 387.7) = 21.03$, $p \leq 0.001$). Within both HB and LB selection groups there were statistically significant differences between strains (Figure 3A and 3B) that were mainly due to the effect of the first 5-trial block, as indicated by the t-test.

3.4 PPI levels of outbred HS rats as a function of reactivity to the pulse-alone trials

In the second part of the study, with the HS rats, we classified the rats according to their %PPI levels with the 50th percentile as the cut-off point to distinguish the low-PPI HS rats from the high-PPI HS rats. Then for each %PPI group, the sample was divided into two halves according to their "startle reactivity" during the BL2 pulse-alone trials.

Firstly, we run a repeated-measures ANOVA with the startle response amplitude to the 5 trial-types as a within-subjects factor and the %PPI selection as between-subjects factors. The "trial-type x PPI selection" interaction ($F (1.3, 443.1) = 26.44$, $p \leq 0.001$) and the "trial-type" effect (F

(1.3, 443.1) = 266.51, p ≤ 0.001) were significant (Figure 4A).For the percentage of prepulse inhibition (%PPI; Figure 4B) we found a significant “trial-type x PPI selection” interaction (F (2.1, 741.4) = 43.82, p ≤ 0.001) along with a “trial-type” effect (F (2.1, 741.4) = 447.73, p ≤ 0.001). The effect of “PPI selection” was also significant (F (1, 346) = 481.01, p ≤ 0.001; Figure 4B).

When taking into account both the “PPI selection” and “startle reactivity” (BL2) selection between-subjects factors, the repeated-measures ANOVA on startle amplitude for the 5 trial types (Figure 4C, 4E) yielded a significant “trial-type x PPI selection x startle reactivity” interaction (F (1.4, 493.4) = 15.19, p ≤ 0.001). The “trial-type x PPI selection” and “trial-type x startle reactivity” interactions were also significant (F (1.4, 493.4) = 40.69, p ≤ 0.001, and F(1.4, 493.4) = 173.33, p ≤ 0.001, respectively). The “trial-type” effect was also significant (F (1.4, 493.4) = 410.18, p ≤ 0.001). Regarding the between-subjects effects, we observed a global significant effect of the “startle reactivity” factor (F(1,344) = 179.31, p ≤ 0.001) on startle amplitude (Figure 4C, 4E). Concerning the %PPI measure (Figure 4D, 4F) we found significant “trial-type x PPI selection” and “trial-type x startle reactivity” interactions (F (2.2, 747.2) = 44.17, p ≤ 0.001, and F(2.2, 747.2) = 4.64, p ≤ 0.008, respectively), along with a “trial-type "effect (F (2.2, 747.2) = 451.36, p ≤ 0.001). The effects of both “PPI selection” and “startle reactivity” main factors were also significant (F (1, 344) = 503.03, p ≤ 0.001, and F(1, 344) = 17.77, p ≤ 0.001, respectively; Figure 4D, 4F).

To better study the above “trial-type x startle reactivity” effects and to elucidate the influence of the “startle reactivity” (BL2) on the PPI measures we conducted separate repeated-measures ANOVA (on “startle amplitude” and on %PPI) for HR and LR groups.

The repeated-measures ANOVA on startle amplitude (in the 5 trial types)of HS rats with high startle reactivity (HS-highPPI-HR and HS-lowPPI-HR groups; Figure 4C) yielded a significant “trial-type x PPI selection” interaction (F(1.4,244) = 27.02 p ≤ 0.001) and a significant “trial-type” effect (F(1.4, 244) = 285.88 p ≤ 0.001) (Figure 4C). The same analysis for the %PPI (Figure 4D) showed a significant “trial-type x PPI selection” interaction (F(1.9,338.3) = 28.36, p ≤ 0.001), a significant “trial-type” effect (F(1.9,338.3) = 251.25, p ≤ 0.001) and a significant effect of the “PPI selection” (F(1,172) = 328.19, p ≤ 0.001; Figure 4D). The results of the t-tests revealed significant differences between both groups in all the measures except in the pulse-alone trials (“PULSE”, in Figure 4C). The differences were also significant in all the prepulse intensities regarding %PPI (Figure 4D)

We run the same analyses for the HS rat sample with low startle reactivity (HS-highPPI-LR and HS-lowPPI-LR groups, figure 4E-F). The repeated measures ANOVA for the startle amplitude in the 5 different trial-types revealed a significant “trial-type x PPI selection” interaction (F(2.2, 385.3) = 65.73, p ≤ 0.001) and a significant “trial-type” effect (F(2.2, 385.3) = 533.67 p ≤ 0.001) (Figure 4E). The same analysis for the %PPI showed a significant “trial-type x PPI selection” interaction (F(2.3,391.4) = 18.40, p ≤ 0.001), a significant “trial-type” (F(2.3,391.4) = 213.85, p≤ 0.001) and a significant effect of the “PPI selection” (F(1,172) = 204.99, p ≤ 0.001; Figure 4F). In the Student’s t-tests, we observed significant differences in the pulse-alone trials (“PULSE”, Figure 4E) and the trials with the two more intense prepulse (PP75+P and PP80+P, Figure 4E). These differences suggest that the high-PPI group shows a greater response to pulse-alone trials and a decreased response in the trials that the pulse is preceded by a prepulse of 75 dB and 80 dB. Furthermore, the differences in %PPI are significant for all the intensities (Figure 4E and 4F).

3.5 Startle response habituation in HS rats stratified for high or low %PPI levels, and influence of the initial baseline startle response amplitude

To see whether there are differences in habituation between the groups selected for differential %PPI scores, we conducted the same ANOVA analysis (with the five 5-trial blocks as the within-subject factor) for the HS rats that were initially selected for their %PPI scores and they were also divided into two groups with high or low baseline startle amplitude ("baseline startle" factor; HB and LB groups, respectively) in the first 5 pulse-alone trials of the session (Figure 5). The results of the repeated-measures ANOVA revealed a significant "trial-block x baseline startle" ($F(3.2,545.5) = 63.89$, $p \leq 0.001$), as well as a significant "trial-block" effect ($F(3.2,545.5) = 90.36$, $p \leq 0.001$) (Figure 5A, 5B).

The separate analyses for each baseline startle group (HB, LB) revealed that in HB rats (Figure 5A) there was a significant "trial-block" effect ($F(3.1, 224.4) = 67.02$, $p \leq 0.001$, Figure 5A). The results of the analysis of LB rats showed a significant "trial-block" effect ($F(2.7, 272.5) = 12.78$, $p \leq 0.001$) and a significant effect of the "PPI selection" ($F(1,100) = 7.01$, $p \leq 0.001$) (Figure 5B).

3.6 Correlations

Finally, a correlational study with all the variables regarding baseline startle and %PPI was carried out. The results are presented in Tables 2-5. Regarding the relation between baseline startle (PULSE) and the percentage of PPI (%PPI65,70,75 and 80), we observed low positive coefficients among these variables, ranging from 0.22 to 0.33 in RHA rats, and from 0.15 to 0.28 in RLA rats. With regard to the correlation coefficients of %PPI and the startle response in each block of five pulse-alone trials, we found low to moderate coefficients in RHA rats, ranging from -0.02 to 0.29 (Table 2). Similarly, in RLA rats these coefficients range from -0.02 to 0.31 (Table 3). In both cases, blocks 3 and 4 of pulse-alone trials showed the higher coefficients as they correspond to the BL2 phase of the test session (see Materials and Methods). We also found moderate negative coefficients between the startle amplitude variables (PP65, 70, 75, 80 +P) and their corresponding %PPI (%PPI65, 70, 75 and 80) with coefficients ranging from -0.27 to -0.40, indicating that larger startle responses are associated with a low percentage of PPI in RHA rats (Table 2). Similarly, in RLA the coefficients ranged from -0.25 to -0.47 (Table 3).

Similar to the Roman rats, in the HS rats selected for their high %PPI, we have found an analogous pattern in the correlation matrix (Table 4), since the correlation coefficients between the PULSE and the %PPI for each prepulse intensity go from 0.16 to 0.27. The correlation coefficients of the %PPI and the startle response in each block of five pulse-alone trials go from -0.15 to 0.44, with the last three blocks showing the strongest relation among variables. Low but significant negative correlation coefficients were also found between the startle amplitude variables (PP65, 70, 75, 80 +P)and their corresponding %PPI variables (from -0.16 to -0.21, Table 3). In contrast, this pattern of correlations was not found in the HS rats exhibiting low levels of %PPI (Table 5).

4. Discussion

The present study shows that, compared to RLAs, the RHA rats display a PPI deficit irrespective of the startle response levels to pulse-alone trials during BL2("PULSE" in Figure 2C, E), although the deficit in %PPI is more evident in rats that exhibit low startle reactivity during the BL2 phase (i.e., RHA-LR vs RLA-LR groups; see Figure 2F compared with Figure 2D). Regarding the results of the "BL2-startle reactivity" measure itself, we have found no differences between the Roman strains in accordance with previous studies from our lab where no significant strain effects were found in that measure, which is used to calculate the %PPI (Esnal et al., 2016; Oliveras et al., 2015; Río-Álamos et al., 2015; Tapias-Espinosa et al., 2019). This is so because startle responses already show clear habituation in both Roman rat strains during the BL2 phase, as is evidenced by the startle amplitude scores of both strains in trials blocks 3-4 in Figure 3A, B.

Concerning the startle habituation process, the present findings agree with previous studies with the Roman rats showing that that RLA rats display higher startle response amplitudes to pulse-alone trials than RHA rats, particularly at the beginning of the session (Aguilar et al., 2000; López-Aumatell et al., 2009ab; Río-Álamos et al., 2015). Most importantly, the present is the first time that a deficit in startle response habituation is shown in RHA vs. RLA rats using a large sample of animals stratified according to their initial baseline startle amplitude (during the first 5-trial block of the session) and showing that irrespective of these initial startle responses RHA rats exhibit a habituation deficit compared with their RLA counterparts (see Figure 3A-B). These findings, along with the robust %PPI deficit of RHA rats, are consistent with previous studies indicating that the RHA strain exhibits schizophrenia-relevant attentional and cognitive deficits in several tasks, as compared to their RLA counterparts and other rat strains/stocks (Esnal et al., 2016; Fernández-Teruel et al., 2006; Martínez-Membrives et al., 2015; Oliveras et al., 2015,2016; Río-Álamos et al., 2019; Tapias-Espinosa et al., 2018, 2019). Thus, the results of RHA rats in PPI and startle response habituation seem to suggest that there is a definite deficit in sensorimotor gating and information processing in that rat strain. This is globally consistent with findings from schizophrenia patients, in which a deficit in habituation of startle has repeatedly been found (in most –though not in all- studies,e.g., Bolino et al., 1994; Geyer et al., 1990; Hammer et al., 2011; Ludewig et al., 2003a, b; Meincke et al.,2004; Mena et al., 2016; Takahashi et al., 2008). It seems that such a habituation deficit might be more commonly found in studies specifically designed to measure habituation rather than those that take habituation measures in the context of a PPI session(e.g., see discussion by Ludewig et al. 2002). Nevertheless, we carried out a correlation analysis (i.e., Pearson's coefficients) between habituation and PPI percentages and we did not observe any significant correlation among these measures in any of the strains(all coefficients ranging from -0.04 to 0.17), suggesting that startle habituation and sensorimotor gating are two different psychophysiological processes.

Secondly, and confirming the above findings from the Roman rats, the HS rats that have been grouped according to their %PPI levels also show PPI differences when they are divided for their BL2 high or low startle reactivity. In fact, the differences between the two selected (HS-High-PPI and HS-Low-PPI) HS groups, regarding both the "startle amplitude" (i.e. PP65+P to PP80+P measures) and %PPI, are maintained when they are further stratified by high (i.e., HR, Figure 4D) or low (i.e., LR, Figure 4F) levels of startle reactivity during the BL2 phase. This, in turn, suggests

that these PPI deficits of RHA and HS-lowPPI rats are genuine alterations of sensorimotor gating processes that are not interfered (at least at the group level) by the startle reactivity levels to pulse-alone trials during the BL2phase.

Several studies have suggested that baseline startle reactivity (during the BL2 phase) can be a confounding factor when interpreting the PPI scores (e.g., Csomor et al., 2008; Hince and Martin-Iverson, 2005; Sandner and Canal, 2007; Scarborough et al., 2019; Shoji and Miyakawa, 2018; Swerdlow et al., 2001; Yee et al., 2004; see also "Introduction"). Csomor et al. (2008) studied in humans the influence of baseline startle dividing the sample into high and low startle groups. They found that the low startle group had higher levels of %PPI and that there was no significant correlation between %PPI and startle response. However, the results were the opposite when the absolute difference of PPI (i.e., the startle amplitude during "prepulse + pulse" trials) was used, meaning that there was a significant positive correlation between the absolute score of PPI and baseline startle at BL2. Remarkably, similar results were found in mice in the same study (Csomor et al., 2008). As mentioned in the "Introduction", factors such as age, strain, housing conditions, and the features of the experimental session may have an important impact on %PPI and startle levels, thus leading to this heterogeneous picture regarding the startle-PPI association. Another relevant factor is the sex of the animals. In fact, regarding the Roman rats, we have observed that adolescent and adult female rats exhibit lower PPI levels than their male counterparts, while adult females also show lower baseline startle responses than adult males (Oliveras et al., submitted). These results are in accordance with previous studies in rats (Lehmann et al., 1999; for review see Hill, 2016) and in humans (Kumari et al., 2004). Additionally, we are currently working on experiments to evaluate whether this low association between startle response and %PPI, found in male Roman rats, is also present in female rats.

In the present study, we have shown that %PPI deficits are present in the RHA rats regardless of the levels of the BL2 startle response (to pulse-alone trials at BL2)they display. Thus, our results are similar to the results from other authors showing that %PPI levels are insensitive to the startle reactivity (BL2) of the animals (Ison et al., 1997). Also supporting this notion, in several pharmacological studies (Oliveras et al., 2017) with the Roman rat strains we found that some drugs either decreased (DOI, apomorphine, haloperidol, clozapine) or increased (dizocilpine) the startle response in pulse-alone trials without affecting strain differences in PPI measures, thus suggesting that in these rats the influence of the startle response to pulse-alone trials on sensorimotor gating is (if any) quite small. In a similar vein, other studies with rats, have shown differences in %PPI with strains exhibiting similar levels of the startle response (for review see Geyer et al., 2001 and Swerdlow et al., 2008, and the references therein).

Moreover, the statistical correlation between both variables is very modest (see Blumenthal et al., 2004; Csomor et al., 2008; Ison et al., 1997; Scarborough et al., 2019; Shoji and Miyakawa, 2018; Yee et al., 2004, but also see Hince and Martin-Iverson, 2005), as also shown in the present study (see Discussion below). So, there is not a clear consensus as to whether the impact of the startle response amplitude on %PPI can be dismissed or, contrarily, researchers have to take it into consideration when interpreting the %PPI results (Csomor et al., 2008; Hince and Martin-Iverson, 2005; Shoji and Miyakawa, 2018).

In the HS study, we have observed that the %PPI differences between the stratified HS-HighPPI and HS-LowPPI subsamples are conserved regardless of the comparison involved HS rats with high (Figure 4C, D) or low(Figure 4E, F) reactivity to the pulse-alone trials during the BL2 phase. These results seem to be comparable to findings by Bast et al. (2001) showing that alterations in startle reactivity are not always accompanied by alterations in PPI and suggest a relative independence between both processes in the HS rats. This in turn is in line with the above-mentioned %PPI findings from RHA vs. RLA rats and is also in accordance with other studies (Bast et al., 2000; Caine et al., 1992; Wan et al., 1996; Zhang et al., 2000). In contrast with the above-mentioned habituation results from RHA vs. RLA rats, we have found no differences in the habituation curves across the five 5-trial blocks of pulse-alone trials between the HS high-baseline (HB, Figure 5A)or low-baseline (LB, Figure 5B) startle subgroups.

To sum up, the results of the present study suggest that the RHA (vs. RLA) rats are a valid model to detect deficits in sensorimotor gating since the reduced levels of %PPI in RHAs are observed regardless of their high or low startle reactivity (to pulse-alone trials) in the BL2 phase. Likewise, the findings from the HS rat study mostly replicate and extend such an independency of %PPI from startle reactivity (to pulse-alone trials) in the BL2 phase, which provides evidence on the generalization of the phenomenon.

Finally, the correlational study shows low associations among the variables measuring startle response amplitude and the percentage of PPI (tables 2-4), whereas there are no significant correlations in the HS-LowPPI rats (Table 5). Therefore, these results overall suggest a predominant but low positive association between the startle response reactivity (at BL2) and %PPI, which is in line with similar low-to-moderate associations we have observed in previous studies using much smaller “n” than the present (Oliveras et al., 2015). Shoji and Miyakawa (2018) also show a positive correlation between both measures, particularly in mice with low startle reactivity. Ison et al. (1997) also carried out a correlational study with mice and rats and they found no significant positive and no significant negative correlations, respectively. Additionally, the authors report that the proportion of shared variance of both measures is less than 10%, similar to findings from most previous reports as well as of the present study.

Thus, there is no consistent evidence across studies regarding the association and/or the direction of this association between startle response reactivity and PPI. Such an inconsistency may depend on different parameters used in the testing procedures, the levels of startle reactivity or %PPI performance, as well as environmental, strain and species (i.e. mice, rats, humans) differences (among others causes), such that the overall predominant picture seems to be that of no association or rather low associations of both signs between these measures (Csomor et al., 2008; Ison et al., 1997; Scarborough et al., 2019; Shoji and Miyakawa, 2018).

In conclusion, the present study shows for the first time that:
1) The PPI deficit of RHA (vs. RLA) rats is present irrespective of the acoustic startle response levels (to pulse-alone trials) of the rats of both strains.
2) HS rats selected for their high or low scores of %PPI show similar differences in this measure when they are grouped by their differential startle amplitude scores (at BL2). Thus, the %PPI impairments found either in RHA and HS-LowPPI rats can be described as sensorimotor gating

deficits, as they are present regardless of the increases or reductions in the startle amplitude to pulse-alone trials during the BL2 phase. It seems important to underscore that this is the first time that such an independency of these responses/measures (i.e., startle amplitude to pulse-alone trials during the BL2 phase and %PPI) is reported on the basis of large samples of these two inbred rat strains and the outbred HS rat stock.

3) Regarding startle habituation, RLA rats habituated to the startling (pulse-alone) stimulus more effectively than RHA rats regardless of the baseline startle responses of the strains. It seems worth highlighting the robustness of these results, based on the use of a large "n"/strain and the within-strain stratification on the basis of the baseline startle during the first 5-trial block. This constitutes a novel finding which suggests a deficit in information processing in RHA rats, and in turn adds to many previous findings indicating that this strain displays a variety of attentional and cognitive impairments (along with many other neurobehavioral phenotypes) that make it a putative model of schizophrenia-relevant features (Esnal et al., 2016; Fernández-Teruel et al., 2006; Giorgi et al., 2019; Martínez-Membrives et al., 2015; Oliveras et al., 2015, 2016, 2017; Río-Álamos et al., 2019; Tapias-Espinosa et al.,2018, 2019). In HS rats, although there seems to be a similar trend for differential habituation between HS-HighPPI-LB and HS-LowPPI-LB (Figure 5B), the "PPI selection x block" interaction did not reach significance, which precludes any conclusion about differential habituation.

4) Regarding the correlation study, we have found low correlational coefficients indicating that we cannot completely rule out the baseline startle response as a lurking variable that has to be controlled in order to measure its influence in PPI studies. However, although such a low association may be true when taking into account individual scores to make correlations, the present study shows that at the group level, and using large rat samples, there seems to be no relevant interference (or influence) between startle response amplitude during BL2 (i.e., the pulse-alone trials used for %PPI calculation) and %PPI levels.

These findings appear to be robust because both large samples and three different strains/stocks of rats (one of them characterized by its high genetic heterogeneity –HS rats-) have been used for the first time. Further studies with similarly large rat samples, and using different rat strains/stocks, might shed light on the generalizability of the present findings.

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5. References

Acri, J.B., Brown, K.J., Saah, M.I., Grunberg, N.E., 1995. Strain and age differences in acoustic startle responses and effects of nicotine in rats. Pharmacol. Biochem. Behav. 50, 191–8.https://doi.org/10.1016/0091-3057(94)00285-q

Al-Amin, H.A., Schwarzkopf, S.B., 1996. Effects of the PCP analog dizocilpine on sensory gating: Potentialrelevance to clinical subtypes of schizophrenia. Biol. Psychiatry 40, 744–754. https://doi.org/10.1016/0006-3223(95)00485-8

Aguilar, R., Gil, L., Tobeña, A., Escorihuela, R.M., Fernández-Teruel, A., 2000. Differential effects of cohort removal stress on the acoustic startle response of the Roman/Verh rat strains. Behav.Genet. 30, 71–75. https://doi.org/10.1023/A:1002042711672

Bast, T., Zhang, W., Feldon, J., White, I.M., 2000. Effects of MK801 and neuroleptics on prepulse inhibition: re-examination in two strains of rats. Pharmacol.Biochem.Behav.67, 647–58.https://doi.org/10.1016/s0091-3057(00)00409-3

Bast, T., Zhang, W.N., Feldon, J., 2001. Hyperactivity, decreased startle reactivity, and disrupted prepulse inhibition following disinhibition of the rat ventral hippocampus by the GABAA receptor antagonist picrotoxin. Psychopharmacology (Berl). 156, 225–233. https://doi.org/10.1007/s002130100775

Blumenthal, T.D., Elden, A., Flaten, M.A., 2004. A comparison of several methods used to quantify prepulse inhibition of eyeblink responding. Psychophysiology 41, 326–332. https://doi.org/10.1111/j.1469-8986.2003.00144.x

Bolino, F., Di Michele, V., Di Cicco, L., Manna, V., Daneluzzo, E., Casacchia, M., 1994. Sensorimotor gating and habituation evoked by electro-cutaneous stimulation in schizophrenia. Biol. Psychiatry 36, 670–679. https://doi.org/10.1016/0006-3223(94)91176-2

Caine, S.B., Geyer, M.A., Swerdlow, N.R., 1992. Hippocampal modulation of acoustic startle and prepulse inhibition in the rat.Pharmacol.Biochem.Behav. 43, 1201–1208. https://doi.org/10.1016/0091-3057(92)90503-8

Csomor, P.A., Vollenweider, F.X., Feldon, J., Yee, B.K., 2005. On the feasibility to detect and to quantify prepulse-elicited reaction in prepulse inhibition of the acoustic startle reflex in humans. Behav. Brain Res. 162, 256–263. https://doi.org/10.1016/j.bbr.2005.03.020

Csomor, P.A., Yee, B.K., Vollenweider, F.X., Feldon, J., Nicolet, T., Quednow, B.B., 2008. On the influence of baseline startle reactivity on the indexation of prepulse inhibition. Behav. Neurosci. 122, 885–900. https://doi.org/10.1037/0735-7044.122.4.885

del Río, C., Oliveras, I., Cañete, T., Blázquez, G., Tobeña, A., Fernández-Teruel, A., 2014. Genetic Rat Models of Schizophrenia-Relevant Symptoms.World J. Neurosci.04, 261–278. https://doi.org/10.4236/wjns.2014.43030

Esnal, A., Sánchez-González, A., Río-Álamos, C., Oliveras, I., Cañete, T., Blázquez, G., Tobeña, A., Fernández-Teruel, A., 2016. Prepulse inhibition and latent inhibition deficits in Roman high-avoidance vs. Roman low-avoidance rats: Modeling schizophrenia-related features. Physiol. Behav. 163, 267–273. https://doi.org/10.1016/j.physbeh.2016.05.020

Feifel, D., 1999. Individual differences in prepulse inhibition of startle as a measure of individual dopamine function. Behav. Neurosci. 113, 1020–1029. https://doi.org/10.1037//0735-7044.113.5.1020

Feifel, D., Priebe, K., Shilling, P.D., 2001. Startleandsensorimotorgating in rats lacking CCK-A receptors. Neuropsychopharmacology 24, 663–670. https://doi.org/10.1016/S0893-133X(00)00235-9

Fernández-Teruel, A., Blázquez, G., Pérez, M., Aguilar, R., Cañete, T., Guitart, M., Giménez-Llort, L., Tobeña, A., 2006. Latent inhibition threshold in Roman high-avoidance rats: A psychogenetic model of abnormalities in attentional filter? Actas Españolas Psiquiatr. 34, 257–263.

Furuya, Y., Kagaya, T., Ogura, H., Nishizawa, Y., 1999. Competitive NMDA receptor antagonists disrupt prepulse inhibition without reduction of startle amplitude in a dopamine receptor-independent manner in mice. Eur. J. Pharmacol. 364, 133–140. https://doi.org/10.1016/S0014-2999(98)00839-5

Geyer, M.A., Swerdlow, N.R., Mansbach, R.S., Braff, D.L., 1990. Startle response models of sensorimotor gating and habituation deficits in schizophrenia. Brain Res. Bull. 25, 485–498. https://doi.org/10.1016/0361-9230(90)90241-Q

Geyer, M.A., Krebs-Thomson, K., Braff, D.L., Swerdlow, N.R., 2001. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology (Berl). 156, 117–154. https://doi.org/10.1007/s002130100811

Giorgi, O., Corda, M.G., Fernández-Teruel, A., 2019. A genetic model of impulsivity, vulnerability to drug abuse and schizophrenia-relevant symptoms with translational potential: The roman high- Vs. Low-avoidance rats. Front. Behav.Neurosci.13, 145.https://doi.org/10.3389/fnbeh.2019.00145

Hammer, T.B., Oranje, B., Fagerlund, B., Bro, H., Glenthøj, B.Y., 2011. Stability of prepulse inhibition and habituation of the startle reflex in schizophrenia: a 6-year follow-up study of initially antipsychotic-naive, first-episode schizophrenia patients. Int. J. Neuropsychopharmacol. 14, 913–925. https://doi.org/10.1017/S1461145711000034

Hansen, C., Spuhler, K., 1984. Development of the National Institutes of Health Genetically Heterogeneous Rat Stock.Alcohol.Clin. Exp. Res. 8, 477–479. https://doi.org/10.1111/j.1530-0277.1984.tb05706.x

Hantsoo, L., Golden, C.E.M., Kornfield, S., Grillon, C., Epperson, C.N., 2018. Startling Differences: Using the Acoustic Startle Response to Study Sex Differences and Neurosteroids in Affective Disorders. Curr. Psychiatry Rep. 20, 40. https://doi.org/10.1007/s11920-018-0906-y

Hince, D.A., Martin-Iverson, M.T., 2005.Differences in prepulse inhibition (PPI) between Wistar and Sprague-Dawley rats clarified by a new method of PPI standardization.Behav.Neurosci. 119, 66–77. https://doi.org/10.1037/0735-7044.119.1.66

Hill, R.A., 2016. Sex differences in animal models of schizophrenia shed light on the underlying pathophysiology. Neurosci.Biobehav. Rev. 67, 41–56. https://doi.org/10.1016/J.NEUBIOREV.2015.10.014

Hoffman, H.S., Ison, J. R., 1992. Reflex modification and the analysis of sensory processing in developmental and comparative research. In B. A. Campbell, H. Hayne, & R. Richardson (Eds.), Attention and information processing in infants and adults: Perspectives from human and animal research.Lawrence Erlbaum Associates, Inc. pp 83–111

Hoffman, H.S., Ison, J.R., 1980. Reflex modification in the domain of startle: I. Some empirical findings and their implications for how the nervous system processes sensory input. Psychol. Rev. 87, 175–189. https://doi.org/10.1037/0033-295X.87.2.175

Ison, J.R., Taylor, M.K., Bowen, G.P., Schwarzkopf, S.B., 1997. Facilitation and inhibition of the acoustic startle reflex in the rat after a momentary increase in background noise level. Behav. Neurosci. 111, 1335–52.https://doi.org/10.1037//0735-7044.111.6.1335

Koch, M., Schnitzler, H.-U., 1997. The acoustic startle response in rats—circuits mediating evocation, inhibition and potentiation. Behav. Brain Res. 89, 35–49. https://doi.org/10.1016/S0166-4328(97)02296-1

Kumari, V., Aasen, I., Sharma, T., 2004. Sex differences in prepulse inhibition deficits in chronic schizophrenia. Schizophr. Res. 69, 219–235. https://doi.org/10.1016/J.SCHRES.2003.09.010.

Lehmann, J., Pryce, C.R., Feldon, J., 1999. Sex differences in the acoustic startle response and prepulse inhibition in Wistar rats. Behav. Brain Res. 104, 113–117. https://doi.org/10.1016/S0166-4328(99)00058-3

Logue, S.F., Owen, E.H., Rasmussen, D.L., Wehner, J.M., 1997. Assessment of locomotor activity, acoustic and tactile startle, and prepulse inhibition of startle in inbred mouse strains and F1 hybrids: Implications of genetic background for single gene and quantitative trait loci analyses. Neuroscience 80, 1075–1086. https://doi.org/10.1016/S0306-4522(97)00164-4

López-Aumatell, R., Blázquez, G., Gil, L., Aguilar, R., Cañete, T., Giménez-Llort, L., Tobeña, A., Fernández-Teruel, A., 2009a. The Roman High- and Low-Avoidance rat strains differ in fear-potentiated startle and classical aversive conditioning. Psicothema 21, 27–32.

López-Aumatell, R., Vicens-Costa, E., Guitart-Masip, M., Martínez-Membrives, E., Valdar, W., Johannesson, M., Cañete, T., Blázquez, G., Driscoll, P., Flint, J., Tobeña, A., Fernández-Teruel, A., 2009b. Unlearned anxiety predicts learned fear: A comparison among heterogeneous rats and the Roman rat strains. Behav. Brain Res. 202, 92–101. https://doi.org/10.1016/j.bbr.2009.03.024

Ludewig, K., Geyer, M.A., Etzensberger, M., Vollenweider, F.X., 2002.Stability of the acoustic startle reflex, prepulse inhibition, and habituation in schizophrenia.Schizophr. Res. 55, 129–137. https://doi.org/10.1016/S0920-9964(01)00198-0

Ludewig, K., Geyer, M.A., Vollenweider, F.X., 2003a. Deficits in prepulse inhibition and habituation in never-medicated, first-episode schizophrenia. Biol. Psychiatry 54, 121–128. https://doi.org/10.1016/S0006-3223(02)01925-X

Ludewig, K., Ludewig, S., Seitz, A., Obrist, M., Geyer, M.A., Vollenweider, F.X., 2003b. The acoustic startle reflex and its modulation: Effects of age and gender in humans. Biol. Psychol. 63, 311–323. https://doi.org/10.1016/S0301-0511(03)00074-7

Martínez-Membrives, E., López-Aumatell, R., Blázquez, G., Cañete, T., Tobeña, A., Fernández-Teruel, A., 2015. Spatial learning in the genetically heterogeneous NIH-HS rat stock and RLA-I/RHA-I rats: Revisiting the relationship with unconditioned and conditioned anxiety. Physiol. Behav. 144, 15–25. https://doi.org/10.1016/j.physbeh.2015.03.003

Meincke, U., Light, G.A., Geyer, M.A., Braff, D.L., Gouzoulis-Mayfrank, E., 2004. Sensitization and habituation of the acoustic startle reflex in patients with schizophrenia. Psychiatry Res. 126, 51–61. https://doi.org/10.1016/j.psychres.2004.01.003

Mena, A., Ruiz-Salas, J.C., Puentes, A., Dorado, I., Ruiz-Veguilla, M., De la Casa, L.G., 2016. Reduced Prepulse Inhibition as a Biomarker of Schizophrenia. Front. Behav.Neurosci. 10, 202. https://doi.org/10.3389/fnbeh.2016.00202

Oliveras, I., Río-Álamos, C., Cañete, T., Blázquez, G., Martínez-Membrives, E., Giorgi, O., Corda, M.G., Tobeña, A., Fernández-Teruel, A., 2015. Prepulse inhibition predicts spatial working memory performance in the inbred Roman high- and low-avoidance rats and in genetically heterogeneous NIH-HS rats: Relevance for studying pre-attentive and cognitive anomalies in schizophrenia. Front. Behav.Neurosci.9, 213.https://doi.org/10.3389/fnbeh.2015.00213

Oliveras, I., Sánchez-González, A., Piludu, M.A., Gerboles, C., Río-Álamos, C., Tobeña, A., Fernández-Teruel, A., 2016. Divergent effects of isolation rearing on prepulse inhibition, activity, anxiety and hippocampal-dependent memory in Roman high- and low-avoidance rats: A putative model of schizophrenia-relevant features. Behav. Brain Res. 314, 6–15. https://doi.org/10.1016/j.bbr.2016.07.047

Oliveras, I., Sánchez-González, A., Sampedro-Viana, D., Piludu, M.A., Río-Alamos, C., Giorgi, O., Corda, M.G., Aznar, S., González-Maeso, J., Gerbolés, C., Blázquez, G., Cañete, T., Tobeña, A., Fernández-Teruel, A., 2017. Differential effects of antipsychotic and propsychotic drugs on prepulse inhibition and locomotor activity in Roman high- (RHA) and low-avoidance (RLA) rats.Psychopharmacology (Berl). 234, 957–975. https://doi.org/10.1007/s00213-017-4534-8

Østerbøg, T.B., On, D.M., Oliveras, I., Río-Álamos, C., Sanchez-Gonzalez, A., Tapias-Espinosa, C., Tobeña, A., González-Maeso, J., Fernández-Teruel, A., Aznar, S., 2020. MetabotropicGlutamate Receptor 2 andDopamine Receptor 2 GeneExpressionPredictSensorimotorGatingResponse in theGeneticallyHeterogeneous NIH-HS Rat Strain. Mol. Neurobiol. 57,1516-1528https://doi.org/10.1007/s12035-019-01829-w

Paylor, R., Crawley, J.N., 1997. Inbredstraindifferences in prepulse inhibition of the mouse startle response. Psychopharmacology (Berl). 132, 169–180. https://doi.org/10.1007/s002130050333

Rankin, C.H., Abrams, T., Barry, R.J., Bhatnagar, S., Clayton, D.F., Colombo, J., Coppola, G., Geyer, M.A., Glanzman, D.L., Marsland, S., McSweeney, F.K., Wilson, D.A., Wu, C.F., Thompson, R.F., 2009. Habituation revisited: An updated and revised description of the behavioral characteristics of habituation. Neurobiol. Learn. Mem. 92, 135–138. https://doi.org/10.1016/j.nlm.2008.09.012

Rigdon, G.C., 1990. Differential effects of apomorphine on prepulse inhibition of acoustic startle reflex in two rat strains. Psychopharmacology (Berl). 102, 419–21.https://doi.org/10.1007/bf02244115

Río-Àlamos, C., Oliveras, I., Cañete, T., Blázquez, G., Martínez-Membrives, E., Tobeña, A., Fernández-Teruel, A., 2015. Neonatal handling decreases unconditioned anxiety, conditioned fear, and improves two-way avoidance acquisition: a study with the inbred Roman high (RHA-I)- and low-avoidance (RLA-I) rats of both sexes. Front. Behav.Neurosci.9, 174.https://doi.org/10.3389/fnbeh.2015.00174

Río-Álamos, C., Gerbolés, C., Tapias-Espinosa, C., Sampedro-Viana, D., Oliveras, I., Sánchez-González, A., Cañete, T., Blázquez, G., Del Mar López, M., Baldellou, C., Otaegui, P.J., Tobeña, A., Fernández-Teruel, A., 2017. Conservation of Phenotypes in the Roman High- andLow-Avoidance Rat StrainsAfterEmbryoTransfer. Behav. Genet. 47, 537–551. https://doi.org/10.1007/s10519-017-9854-2

Río-Álamos, C., Piludu, M.A., Gerbolés, C., Barroso, D., Oliveras, I., Sánchez-González, A., Cañete, T., Tapias-Espinosa, C., Sampedro-Viana, D., Torrubia, R., Tobeña, A., Fernández-Teruel, A., 2019. Volumetric brain differences between the Roman rat strains: Neonatal handling effects, sensorimotor gating and working memory. Behav. Brain Res. 361, 74–85. https://doi.org/10.1016/j.bbr.2018.12.033

Sampedro-Viana, D., Cañete, T., Sanna, F., Soley, B., Giorgi, O., Corda, M.G., Torrecilla, P., Oliveras, I., Tapias-Espinosa, C., Río-Álamos, C., Sánchez-González, A., Tobeña, A., Fernández-Teruel, A., 2021. Decreased social interaction in the RHA rat model of schizophrenia-relevantfeatures: Modulationby neonatal handling. Behav. Processes 188, 104397. https://doi.org/10.1016/j.beproc.2021.104397

Sánchez-González, A., Esnal, A., Río-Álamos, C., Oliveras, I., Cañete, T., Blázquez, G., Tobeña, A., Fernández-Teruel, A., 2016. Association between prepulse inhibition of the startle response and latent inhibition of two-way avoidance acquisition: A study with heterogeneous NIH-HS rats. Physiol. Behav. 155, 195–201. https://doi.org/10.1016/j.physbeh.2015.12.011

Sánchez-González, A., Oliveras, I., Río-Álamos, C., Piludu, M.A., Gerbolés, C., Tapias-Espinosa, C., Tobeña, A., Aznar, S., Fernández-Teruel, A., 2020. Dissociation between schizophrenia-relevant behavioral profiles and volumetric brain measures after long-lasting social isolation in Roman rats.Neurosci. Res. 155, 43–55. https://doi.org/10.1016/j.neures.2019.07.002

Sánchez-González, A., Thougaard, E., Tapias-Espinosa, C., Cañete, T., Sampedro-Viana, D., Saunders, J.M., Toneatti, R., Tobeña, A., Gónzalez-Maeso, J., Aznar, S., Fernández-Teruel, A., 2021.Increased thin-spine density in frontal cortex pyramidal neurons in a genetic rat model of schizophrenia-relevant features. Eur. Neuropsychopharmacol. 44, 79–91. https://doi.org/10.1016/j.euroneuro.2021.01.006

Sandner, G., Canal, N.M., 2007. Relationship between PPI and baseline startle response. Cogn. Neurodyn. 1, 27–37. https://doi.org/10.1007/s11571-006-9008-3

San-Martin, R., Castro, L.A., Menezes, P.R., Fraga, F.J., Simões, P.W., Salum, C., 2020. Meta-Analysis of Sensorimotor Gating Deficits in Patients With Schizophrenia Evaluated by Prepulse Inhibition Test. Schizophr. Bull. 46, 1–16. https://doi.org/10.1093/schbul/sbaa059

Scarborough, J., Mueller, F., Weber-Stadlbauer, U., Richetto, J., Meyer, U., 2019.Dependency of prepulse inhibition deficits on baseline startle reactivity in a mouse model of the human 22q11.2 microdeletion syndrome. Genes, Brain Behav. 18, e12523.https://doi.org/10.1111/gbb.12523

Schwegler, H., Pilz, P.K.D., Koch, M., Fendt, M., Linke, R., Driscoll, P., 1997.The acoustic startle response in inbred Roman high- and low-avoidance rats.Behav.Genet. 27, 579–582. https://doi.org/10.1023/A:1021465217299

Shoji, H., Miyakawa, T., 2018. Relationships between the acoustic startle response and prepulse inhibition in C57BL/6J mice: A large-scale meta-analytic study. Mol. Brain 11, 42. https://doi.org/10.1186/s13041-018-0382-7

Swerdlow, N.R., Braff, D.L., Geyer, M.A., Koob, G.F., 1986. Central dopamine hyperactivity in rats mimics abnormal acoustic startle response in schizophrenics. Biol. Psychiatry 21, 23–33. https://doi.org/10.1016/0006-3223(86)90005-3

Swerdlow, N.R., Mansbach, R.S., Geyer, M.A., Pulvirenti, L., Koob, G.F., Braff, D.L., 1990. Amphetaminedisruption of prepulse inhibition of acoustic startle is reversed by depletion of mesolimbic dopamine. Psychopharmacology (Berl). 100, 413–416. https://doi.org/10.1007/BF02244616

Swerdlow, N.R., Geyer, M.A., Braff, D.L., 2001. Neural circuit regulation of prepulse inhibition of startle in the rat: Current knowledge and future challenges. Psychopharmacology (Berl). 156,194-215. https://doi.org/10.1007/s002130100799

Swerdlow, N.R., Weber, M., Qu, Y., Light, G.A., Braff, D.L., 2008. Realistic expectations of prepulse inhibition in translational models for schizophrenia research.Psychopharmacology (Berl).199,331–388.https://doi.org/10.1007/s00213-008-1072-4

Swerdlow, N.R., Braff, D.L., Geyer, M.A., 2016. Sensorimotor gating of the startle reflex: What we said 25 years ago, what has happened since then, and what comes next. J. Psychopharmacol. 30, 1072-1081. https://doi.org/10.1177/0269881116661075

Swerdlow, N.R., Light, G.A., 2016. Animal models of deficient sensorimotor gating in schizophrenia: Are they still relevant? Curr.Top.Behav.Neurosci. 28, 305–325. https://doi.org/10.1007/7854_2015_5012

Takahashi, H., Iwase, M., Ishii, R., Ohi, K., Fukumoto, M., Azechi, M., Ikezawa, K., Kurimoto, R., Canuet, L., Nakahachi, T., Iike, N., Tagami, S., Morihara, T., Okochi, M., Tanaka, T., Kazui, H., Yoshida, T., Tanimukai, H., Yasuda, Y., Kudo, T., Hashimoto, R., Takeda, M., 2008. Impaired prepulse inhibition and habituation of acoustic startle response in Japanese patients with schizophrenia. Neurosci. Res. 62, 187–194. https://doi.org/10.1016/j.neures.2008.08.006

Tapias-Espinosa, C., Río-Álamos, C., Sampedro-Viana, D., Gerbolés, C., Oliveras, I., Sánchez-González, A., Tobeña, A., Fernández-Teruel, A., 2018. Increased exploratory activity in rats with deficient sensorimotor gating: a study of schizophrenia-relevant symptoms with genetically heterogeneous NIH-HS and Roman rat strains.Behav. Processes 151, 96–103. https://doi.org/10.1016/j.beproc.2018.03.019

Tapias-Espinosa, C., Río-Álamos, C., Sánchez-González, A., Oliveras, I., Sampedro-Viana, D., Castillo-Ruiz, M. del M., Cañete, T., Tobeña, A., Fernández-Teruel, A., 2019. Schizophrenia-like reduced sensorimotor gating in intact inbred and outbred rats is associated with decreased medial prefrontal cortex activity and volume. Neuropsychopharmacology 44, 1975–1984. https://doi.org/10.1038/s41386-019-0392-x

Venables, P.H., 1966. Psychophysiological aspects of schizophrenia.Br. J. Med. Psychol. 39, 289–297. https://doi.org/10.1111/j.2044-8341.1966.tb00979.x

Wan, F.-J., Caine, S.B., Swerdlow, N.R., 1996. The ventral subiculum modulation of prepulse inhibition is not mediated via dopamine D2 or nucleus accumbens non-NMDA glutamate receptor activity. Eur. J. Pharmacol. 314, 9–18. https://doi.org/10.1016/S0014-2999(96)00535-3

Yee, B.K., Russig, H., Feldon, J., 2004. Apomorphine-induced prepulse inhibition disruption is associated with a paradoxical enhancement of prepulse stimulus reactivity. Neuropsychopharmacology 29, 240–8.https://doi.org/10.1038/sj.npp.1300323

Zhang, W.-N., Bast, T., Feldon, J., 2000. Microinfusion of the non-competitive N-methyl-d-aspartate receptor antagonist MK-801 (dizocilpine) into the dorsal hippocampus of Wistar rats does not affect latent inhibition and prepulse inhibition, but increases startle reaction and locomotor activity. Neuroscience 101, 589–599. https://doi.org/10.1016/S0306-4522(00)00418-8

Figure Legends

Figure 1.-The structure of the PPI session (24 minutes) is represented. Following 5 min of habituation to the box, the session can be divided into three parts or phases. In the first part (BL1), 10 pulse-alone trials were presented to establish a baseline startle response. In the second part (Phase 2), pulse-alone (BL2) and prepulse + pulse trials (PPI phase) were delivered randomly. Finally, in the third part of the session,5 pulse-alone trials were delivered. * The mean of the scores of the first 5 pulse-alone trials (5-trial block 1) were used to divide the sample into two halves (50th percentile; named High or Low baseline; HB, LB). ** The mean of the scores of the pulse–alone trials of this part (5-trial blocks 3 and 4, i.e., BL2) were used to divide the sample into two halves (the cut-off point was the 50th percentile; named High or Low reactivity, HR, LR).

Figure 2.- A) Mean ± SEM of startle amplitude in pulse-alone (PULSE; BL2 phase) trials, as well as the startle amplitude in PP65+Pulse to PP80+Pulse (PP65+P to PP80+P) trials in the Roman rats. **B)** Mean ± SEM of the percentage of PPI in "Prepulse + Pulse" trials at different prepulse intensities for the Roman rats. **C)** Mean ± SEM of startle amplitude in pulse-alone trials, as well as the startle amplitude in PP65+Pulse to PP80+Pulse trials in the Roman rats with high reactivity to pulse alone trials. **D)** Mean ± SEM of the percentage of PPI in "Prepulse + Pulse" trials at different prepulse intensities in the Roman rats with high reactivity to pulse alone trials. **E)** Mean ± SEM of startle amplitude in pulse-alone trials, as well as the startle amplitude in PP65+Pulse to PP80+Pulse trials in the Roman rats with low reactivity to pulse alone trials. **F)** Mean ± SEM of the percentage of PPI in "Prepulse + Pulse" trials at different prepulse intensities in the Roman rats with low reactivity to pulse alone trials. *p < 0.05, **p<0.01, ***p<0.001, "group x trial-type" effect (repeated measures ANOVA). ### p<0.001 "strain" effect (repeated measures ANOVA). $^{\&}$Adjusted p<0.001 significant t-tests.

Figure 3.- A) Mean ± SEM of startle amplitude of the Roman rats with high baseline startle responses during the first 5 pulse-alone trials of the session. **B)** Mean ± SEM of startle amplitude of the Roman rats with low baseline startle responses during the first 5 pulse-alone trials of the session. ***p<0.001, "group x trial- block" effect (repeated measures ANOVA). $^{\#\#\#}$ p<0.001 "strain" effect (repeated measures ANOVA).$^{\&}$ p<0.001, statistically significant t-test.

Figure 4.- A) Mean ± SEM of startle amplitude in pulse-alone trials, as well as the startle amplitude in PP65+Pulse to PP80+Pulse trials in the HS selected for their extreme %PPI values. **B)** Mean ± SEM of the percentage of PPI in "Prepulse + Pulse" trials at different prepulse intensities in the HS selected for their extreme %PPI values. **C)** Mean ± SEM of startle amplitude in pulse-alone trials, as well as the startle amplitude in PP65+Pulse to PP80+Pulse trials in HS rats with high reactivity in pulse-alone trials. **D)** Mean ± SEM of the percentage of PPI in "Prepulse + Pulse" trials at different prepulse intensities in HS rats with high reactivity in pulse-alone trials. **E)** Mean ± SEM of startle amplitude in pulse-alone trials, as well as the startle amplitude in PP65+Pulse to PP80+Pulse trials in HS rats with high reactivity in pulse-alone trials ((n = 87 for both groups)). **F)** Mean ± SEM of the percentage of PPI in "Prepulse + Pulse" trials at different prepulse intensities in HS rats with high reactivity in pulse-alone trials. ***p<0.001, "trial-type x PPI selection x baseline selection" or "trial-type x PPI selection" effect;).$^{\#\#\#}$ p<0.001 "PPI selection" effect (repeated measures ANOVA).$^{\&}$Adjusted p-value<0.001 significant t-test.

Figure 5.- A) Mean ± SEM of startle amplitude of HS rats with high baseline startle responses during the first 5 pulse-alone trials of the session. **B)** Mean ± SEM of startle amplitude of HS rats with low baseline startle responses during the first 5 pulse-alone trials of the session. $^{\#\#}$ p<0.01 "PPI selection" effect (repeated measures ANOVA).

Table legends

Table 1.- Mean (± SEM) of the main variables of the PPI session. BL1: startle response in the first 10 pulse-alone trials. BL2: startle response in the 10 pulse alone trials that were randomly presented throughout the PPI phase (see "2.2 Prepulse inhibition" section). BL3: startle response in the last 5 pulse-alone trials delivered at the end of the PPI session. %PPI65-%PPI80: percentage of PPI for the four prepulse intensities (65-80 dB). TOTAL %PPI: percentage of PPI averaged across the four prepulse intensities.a,b : $p<0.001$ between groups with the same letter Student's t-tests.

Table 2.-Pearson's correlations coefficients among the main variables of the PPI session for the RHA rats. PULSE: startle response in the 10 pulse alone trials that were randomly presented throughout the PPI phase(corresponds to the BL2 phase). PP65+P-PP80+P: startle response amplitude in the trials where the "prepulse (65-80 dB) + pulse" was presented.%PP65-%PPI80: percentage of PPI for the four prepulse intensities (65-80 dB). PULSE BLOCK 1- PULSE BLOCK 5: Startle response in pulse-alone trials grouped in 5-trial blocks across the whole PPI session. *, $p < 0.05$; **, $p < 0.01$.

Table 3.-Pearson's correlations coefficients among the main variables of the PPI session for the RLA rats. Abbreviations and symbols as in Table 2

Table 4.-Pearson's correlations coefficients among the main variables of the PPI session for the HS rats with high levels of PPI. Abbreviations and symbols as in Table 2

Table 5.- Pearson's correlations coefficients among the main variables of the PPI session for the HS rats with low levels of PPI.Abbreviations and symbols as in Table 2.

Figure 1

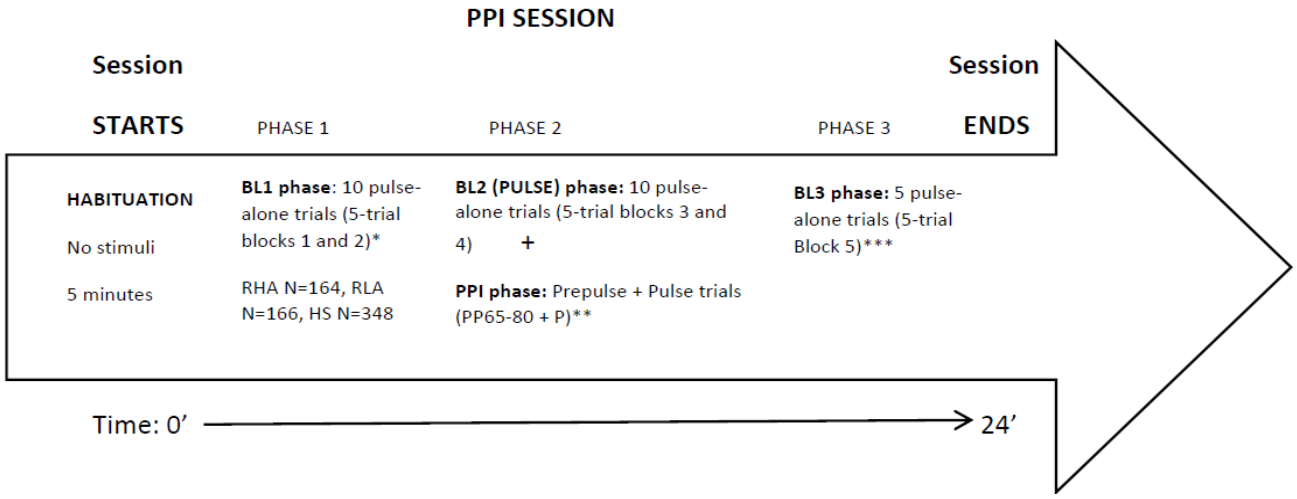
PPI SESSION
Session STARTS
PHASE 1
PHASE 2
PHASE 3
Session ENDS
HABITUATION
No stimuli
5 minutes
BL1 phase: 10 pulse-alone trials (5-trial blocks 1 and 2)*
RHA N=164, RLA N=166, HS N=348
BL2 (PULSE) phase: 10 pulse-alone trials (5-trial blocks 3 and 4) +
PPI phase: Prepulse + Pulse trials (PP65-80 + P)**
BL3 phase: 5 pulse-alone trials (5-trial Block 5)***
Time: 0'
24'

Figure 2

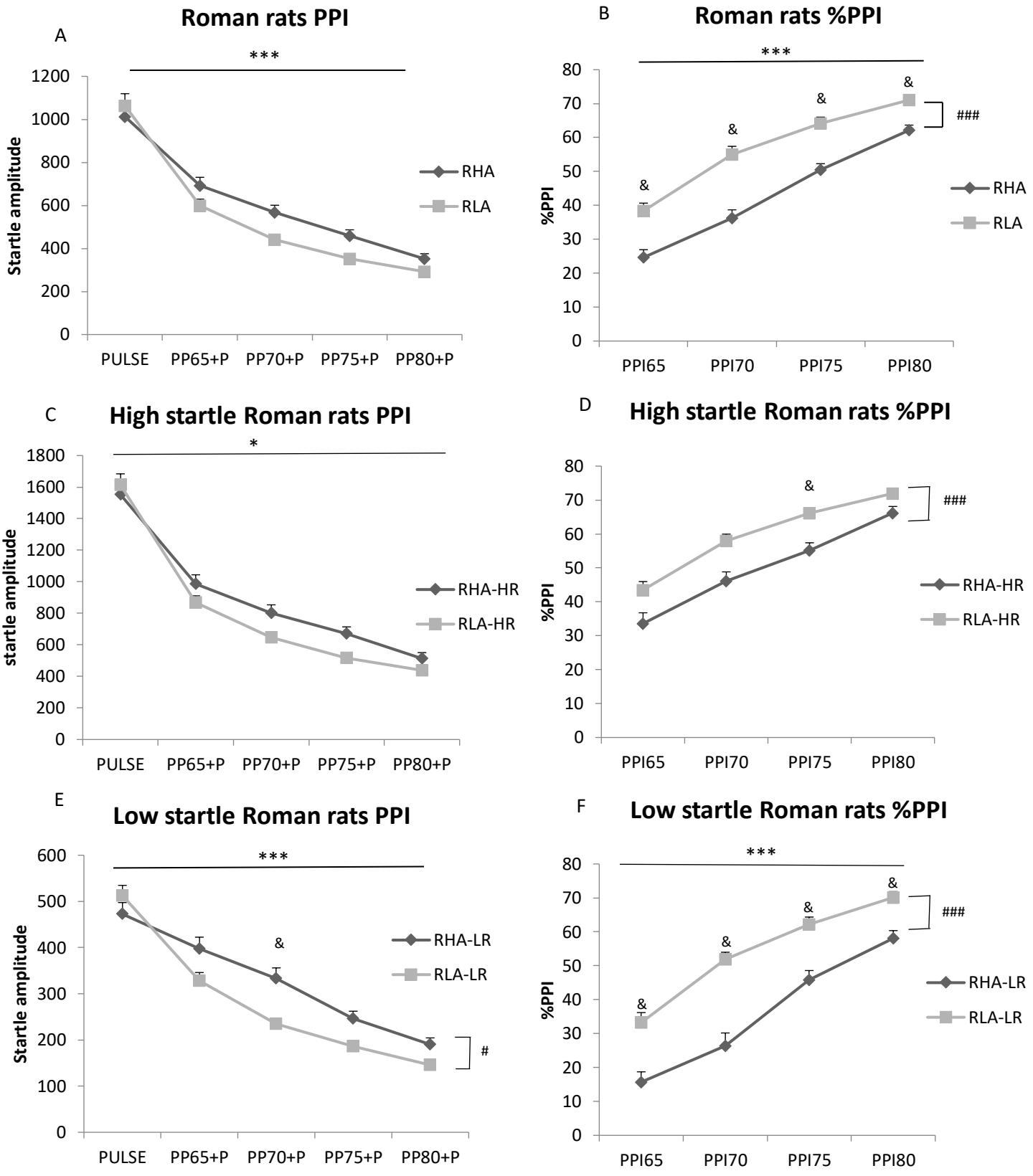
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Figure 3

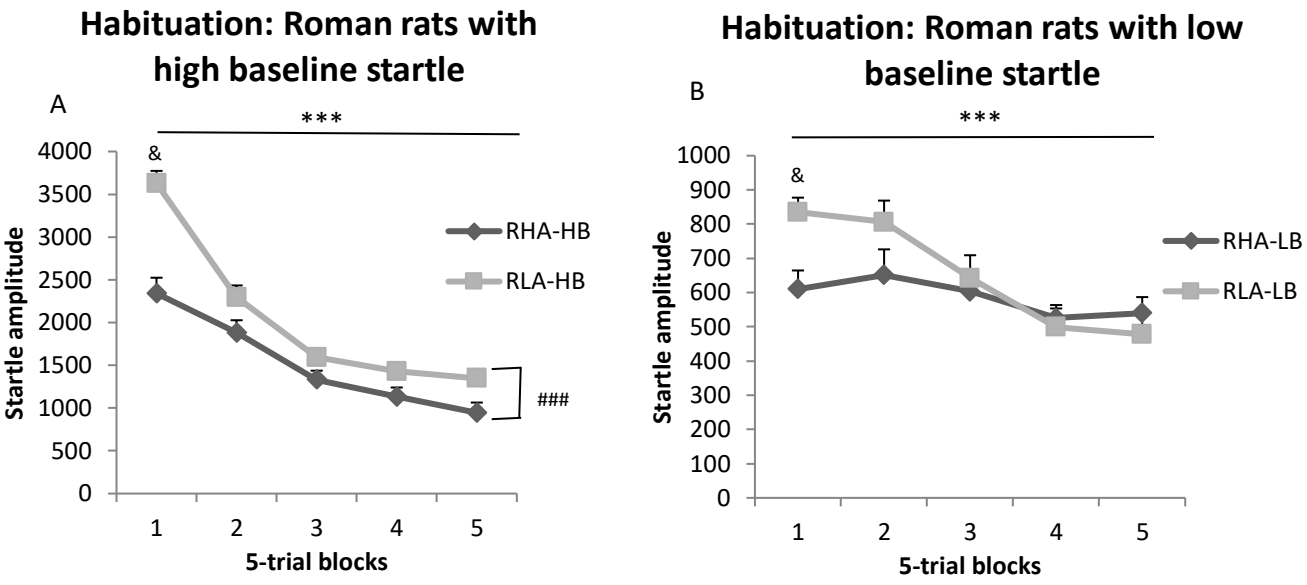
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Figure 4

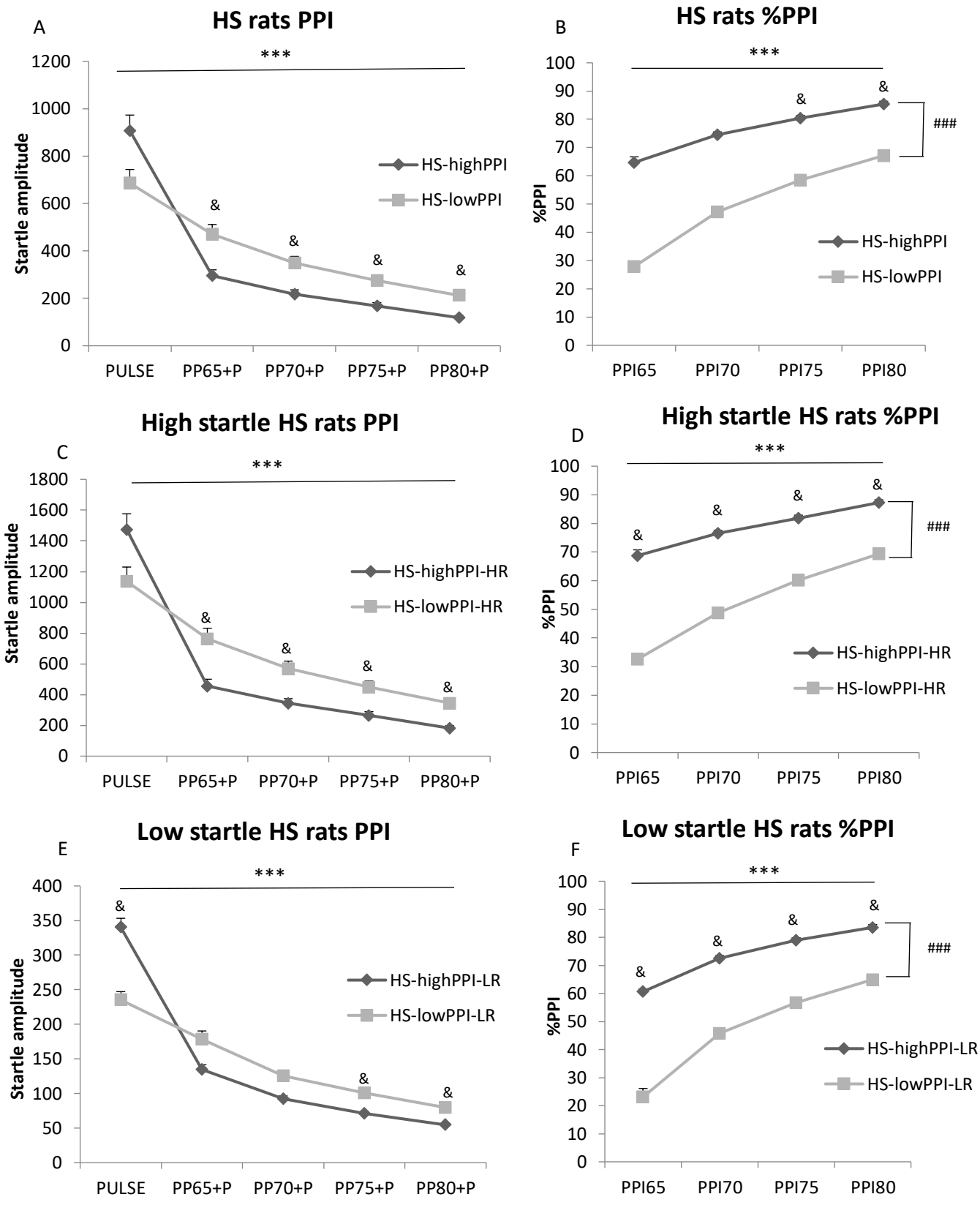
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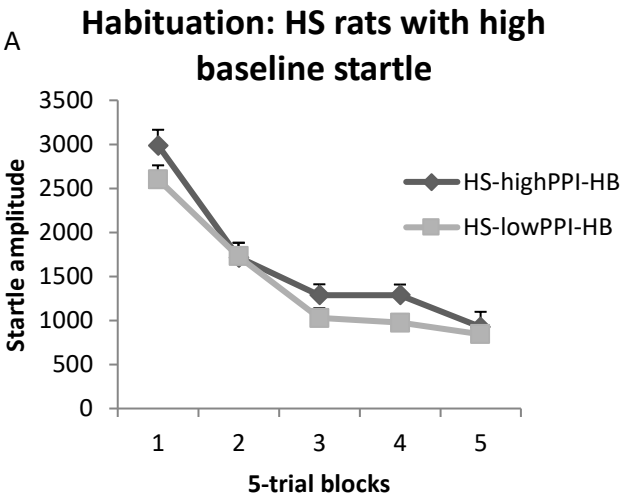
Figure 5

A


Habituation: HS rats with high baseline startle
Startle amplitude
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5-trial blocks
HS-highPPI-HB
HS-lowPPI-HB

B

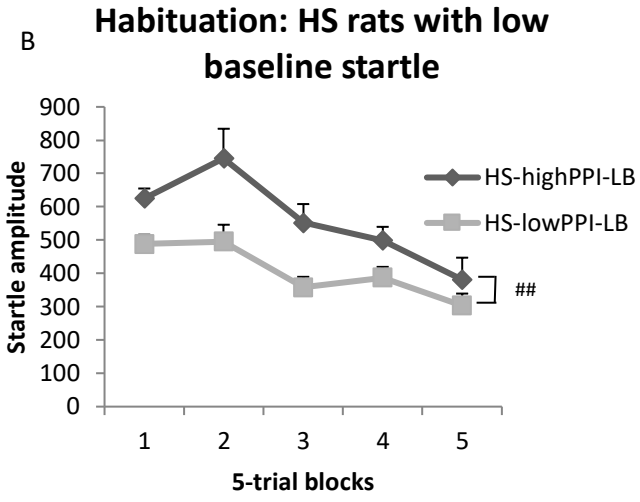
Habituation: HS rats with low baseline startle
Startle amplitude
900
800
700
600
500
400
300
200
100
0
1
2
3
4
5
5-trial blocks
HS-highPPI-LB
HS-lowPPI-LB
##

Table 1

	RHA (n=164)	RLA (n=166)	HS (n=348)
BL1	1557.22 (91.03)	1866.06 (100.39) [a]	1424.08 (70.75)[a]
BL2	1012.70 (58.90)	1062.87 (56.60) [a]	796.91 (44.68) [a]
BL3	742.75 (50.86)	912.49 (74.75) [a]	562.48 (47.37) [a]
%PPI65	24.64 (2.30) [a,b]	38.36 (1.96) [a]	46.29 (1.49)[b]
%PPI70	36.20 (2.49) [a,b]	54.93 (1.45) [a]	60.91 (1.05)[b]
%PPI75	50.46 (1.83) [a,b]	64.18 (1.33) [a]	69.42 (0.87)[b]
%PPI80	62.14 (1.51) [a,b]	71.04 (1.16) [a]	76.29 (0.74) [a,b]
TOTAL %PPI	43.36 (1.82) [a,b]	57.13 (1.29) [a]	63.22 (0.92) [a,b]

Table 2

RHA	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.- PULSE	1													
2.- PP65+P	0.72**	1												
3.- PP70+P	0.64**	0.90**	1											
4.- PP75+P	0.67**	0.90**	0.92**	1										
5.- PP80+P	0.69**	0.89**	0.88**	0.93**	1									
6.- %PPI65	0.33**	-0.28**	-0.23**	-0.19*	-0.19*	1								
7.- %PPI70	0.34**	-0.09	-0.27**	-0.01	-0.01	0.73**	1							
8.- %PPI75	0.27**	-0.18*	-0.25**	-0.34**	-0.28**	0.73**	0.71**	1						
9.- %PPI80	0.22**	-0.21**	-0.26**	-0.30**	-0.40**	0.73**	0.71**	0.84**	1					
10.- PULSE BLOCK 1	0.62**	0.50**	0.45**	0.52**	0.52**	0.02	0.22**	0.08	0.02	1				
11.- PULSE BLOCK 2	0.67**	0.58**	0.51**	0.60**	0.58**	0.09	0.20*	0.02	-0.02	0.79**	1			
12.- PULSE BLOCK 3	0.95**	0.76**	0.69**	0.71**	0.68**	0.22**	0.29**	0.19*	0.02	0.54**	0.65**	1		
13.- PULSE BLOCK 4	0.93**	0.78**	0.72**	0.75**	0.75**	0.18*	0.24**	0.01	0.01	0.63**	0.61**	0.78**	1	
14.- PULSE BLOCK 5	0.83**	0.68**	0.59**	0.59**	0.58**	0.02	0.21*	0.05	0.02	0.48**	0.58**	0.77**	0.80**	1

Table 3

RLA	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.- PULSE	1													
2.- PP65+P	0.78**	1												
3.- PP70+P	0.72**	0.89**	1											
4.- PP75+P	0.69**	0.86**	0.92**	1										
5.- PP80+P	0.72**	0.82**	0.88**	0.90**	1									
6.- %PPI65	0.28**	-0.25**	-0.17*	-0.16*	-0.07	1								
7.- %PPI70	0.21**	-0.25**	-0.42**	-0.34**	-0.18*	0.77**	1							
8.- %PPI75	0.17*	-0.23**	-0.31**	-0.46**	-0.22**	0.70**	0.77**	1						
9.- %PPI80	0.15	-0.19*	-0.27**	-0.32**	-0.47**	0.55**	0.66**	0.67**	1					
10.- PULSE BLOCK 1	0.73**	0.62**	0.59**	0.60**	0.59**	0.20*	0.18*	0.05	0.04	1				
11.- PULSE BLOCK 2	0.72**	0.64**	0.67**	0.69**	0.64**	0.19*	0.11	-0.03	-0.00	0.76**	1			
12.- PULSE BLOCK 3	0.93**	0.74**	0.72**	0.72**	0.67**	0.31**	0.25**	0.16	0.16	0.65**	0.69**	1		
13.- PULSE BLOCK 4	0.93**	0.73**	0.71**	0.71**	0.71**	0.29**	0.23**	0.15	0.11	0.71**	0.66**	0.73**	1	
14.- PULSE BLOCK 5	0.72**	0.62**	0.60**	0.60**	0.63**	0.19*	0.16	0.06	-0.02	0.59**	0.56**	0.61**	0.73**	1

Table 4

HSHIGH PPI	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.- PULSE	1													
2.- PP65+P	0.86**	1												
3.- PP70+P	0.88**	0.86**	1											
4.- PP75+P	0.88**	0.91**	0.87**	1										
5.- PP80+P	0.82**	0.84**	0.88**	0.90**	1									
6.- %PPI65	0.22**	-0.16*	0.04	0.04	0.03	1								
7.- %PPI70	0.16*	-0.02	-0.21**	-0.01	-0.10	0.47**	1							
8.- %PPI75	0.17*	-0.02	-0.02	-0.19*	-0.11	0.33**	0.40**	1						
9.- %PPI80	0.27**	0.10	0.04	0.04	-0.18*	0.29**	0.43**	0.53**	1					
10.- PULSE BLOCK 1	0.59**	0.49**	0.61**	0.57**	0.58**	0.15*	-0.03	-0.15	0.01	1				
11.- PULSE BLOCK 2	0.72**	0.60**	0.70**	0.61**	0.62**	0.18*	0.06	0.12	0.20**	0.63**	1			
12.- PULSE BLOCK 3	0.95**	0.81**	0.82**	0.85**	0.75**	0.22**	0.14	0.14	0.27**	0.57**	0.71**	1		
13.- PULSE BLOCK 4	0.94**	0.81**	0.83**	0.81**	0.79**	0.20**	0.17*	0.17*	0.23**	0.54**	0.64**	0.78**	1	
14.- PULSE BLOCK 5	0.70**	0.46**	0.49**	0.46**	0.49**	0.44**	0.32**	0.32**	0.26*	0.41**	0.73**	0.58**	0.73**	1

Table 5

HS LOW PPI	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.- PULSE	1													
2.- PP65+P	0.93**	1												
3.- PP70+P	0.95**	0.91**	1											
4.- PP75+P	0.93**	0.93**	0.95**	1										
5.- PP80+P	0.90**	0.84**	0.95**	0.93**	1									
6.- %PPI65	0.12	-0.11	0.04	0.02	0.07	1								
7.- %PPI70	0.10	0.01	-0.11	-0.04	-0.05	0.43**	1							
8.- %PPI75	0.09	-0.00	-0.04	-0.16*	-0.09	0.48**	0.59**	1						
9.- %PPI80	0.12	0.08	0.00	-0.03	-0.17*	0.34**	0.44**	0.61**	1					
10.- PULSE BLOCK 1	0.72**	0.67**	0.68**	0.67**	0.68**	0.03	0.07	0.02	-0.01	1				
11.- PULSE BLOCK 2	0.75**	0.75**	0.76**	0.71**	0.73**	-0.01	0.05	0.09	-0.00	0.74**	1			
12.- PULSE BLOCK 3	0.97**	0.90**	0.94**	0.93**	0.90**	0.11	0.08	0.08	0.11	0.70**	0.78**	1		
13.- PULSE BLOCK 4	0.96**	0.89**	0.88**	0.87**	0.82**	0.12	0.11	0.10	0.13	0.69**	0.64**	0.85**	1	
14.- PULSE BLOCK 5	0.77**	0.72**	0.79**	0.68**	0.74**	0.09	0.03	0.14	0.09	0.62**	0.72**	0.74**	0.75**	1