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Brain adaptation to acute stress : effect of time, social buffering and nicotinic cholinergic system

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Running title: prefrontal cortex plasticity after stress and social interaction

Abstract

Social behavior and stress responses both rely on activity of the prefrontal cortex (PFC) and the basolateral nucleus of the amygdala (BLA), and on cholinergic transmission. We previously showed in adult C57BL/6J (B6) that social interaction has a buffering effect on stress-related prefrontal activity, depending on the $\beta2^{-/-}$ cholinergic nicotinic receptors (nAChRs, $\beta2^{-/-}$ mice). The latency for this buffer to emerge being short, we question here whether the associated brain plasticity, reflected by regional c-fos protein quantification and PFC-BLA functional connectivity, is modulated by time. Overall, we show that time normalized the stress-induced PFC hyper-activation in B6 mice and PFC hypo-activation in $\beta2^{-/-}$ mice, with no effect on BLA. It also triggered a multitude of functional links between PFC subareas, and between PFC and BLA in B6 mice but not $\beta2^{-/-}$ mice, showing a central role of nAChRs in this plasticity. Coupled to social interaction and time stress led to novel and drastic diminution of functional connectivity within the PFC in both genotypes. Thus, time, emotional state, and social behavior induced dissociated effects on PFC and BLA activity, and important cortico-cortical reorganizations. Both activity and plasticity were under the control of the β2-nAChRs.

Keywords: Acute stress, amygdala, c-fos protein, prefrontal cortex, time

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Social interactions involve high adaptive flexible behaviors that require the integration of internal and external cues to make coherent decisions in specific environmental contexts. Social interactions, altered in numerous psychiatric disorders, imply emotional and motivational processes and widely engage the prefrontal cortex (PFC) and the amygdala, in particular the basolateral nucleus of the amygdala (BLA) (see Showers and Cantor 1985; Adolphs 2001, 2009; Dalgleish 2004; Insel and Fernald 2004; Bachevalier et Loveland 2006; Lupien et al. 2009; Pessoa 2010; Stanley and Adolphs 2013; Adolphs and Anderson 2013; Bickart et al. 2014; Bicks et al. 2015; Janak and Tye 2015; Demolliens et al. 2017; for reviews). Besides the classical involvement of the autonomic nervous system, the hypothalamic-pituitary-adrenal axis and the hippocampus in stress responses, the PFC and the amygdala are also stress-related brain areas (Buijs et Van Eden 2000; McEwen 2007; Cerqueira et al. 2008; Holmes et Wellman 2009; Lupien et al. 2009; Mora et al. 2012; McEwen et al. 2016; Negrón-Oyarzo et al. 2016; Atrooz et al. 2019; for reviews). Among brain neuromodulators, acetylcholine plays a major role in social interactions. Brain cholinergic transmission mediated by nAChRs is also mainly involved in healthy and pathological cognition (Graef et al. 2011; Picciotto et al. 2012; Hurst et al. 2013; Levin 2013; Mineur et al. 2016; Prado et al. 2017; Muramatsu et al. 2018; Záborszky et al. 2018) and in stress regulation (Mark et al. 1996; Mora et al. 2012; Paul et al. 2015; Picciotto et al. 2015). Notably, the β2, β4, and α7 subunits of the neuronal nicotinic cholinergic receptors (nAChRs) are broadly involved in social behavior (Avale et al. 2011, Salas et al. 2013; Potasiewicz et al. 2017, Nosjean et al. 2018).

We previously performed exhaustive analyses of social behavior and brain activity in C57BL/6J mice (B6 mice) and in mice lacking the β2 subunit of nicotinic receptors ($\beta2^{-/-}$ mice) using a social task that includes an exploratory phase followed by a social interaction phase to promote both novelty exploration and social investigations (Granon et al. 2003; Avale et al. 2011; De Chaumont et al. 2012; Nosjean et al. 2015, 2018). We showed that acute stress

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deteriorated social flexibility (Nosjean et al. 2015) and that social interaction modulated by the presence of β2-nAChRs, played a buffering role on stress-induced brain activation (Nosjean et al. 2018).

In the latter study, we quantified the regional expression of the protein c-fos to reflect brain activation. C-fos protein expression not only depends on the intensity and the duration of the stimulus, but also on the time-course of the protein translation (Honkaniemi et al. 1992; Chowdury et al. 2000; Viau and Sawchenko 2002; Muigg et al. 2009; see also Senba and Ueyama 1997; Kovács 1998, 2008; as reviews). In our previous experiments, the protein expression was measured 90 min after the beginning of stress exposure, i.e., immediately at the end of the social interaction, a delay which falls within the period of peak production of the protein (see Morgan and Curran 1989, Hoffman et al. 1993; Hughes and Dragunow 1995; Kovacs 1998, 2008; Bisler et al. 2002; Guzowski et al. 2005). It was therefore surprising that only an 8-minute social interaction induced significant and specific brain activation, as a very limited time was left for c-fos protein expression. Thus, in the present study, we question whether PFC and amygdala activity evolve with time by adding a 90 min delay after the social task to allow maximal expression of the protein. To this end, B6 and $\beta2^{-/-}$ mice were sacrificed 90 min after either stress alone or after stress followed by the social task. Non-stressed mice were also sacrificed 90 min after the social task.

Materials and Methods

Part of the data used here (all data referring to "short delay") were previously published (Nosjean et al. 2018) as they assess the immediate effect of stress combined or not with a social task on brain activity. As the purpose of the current paper was to compare the effect of a long delay after stress with or without social interactions, to that of a short delay, we included this

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set of data in our statistical comparisons, in addition to corresponding control data (Fig. 1). This was made possible by the fact that our experiments were conducted in the very same environment and in order to reduce the total number of animals.

Animals

Experiments were conducted with male B6 mice (n = 124) and mice lacking the β2 subunit of the nAchRs ($\beta2^{-/-}$, n = 50). At their arrival to animal facilities, 10-11 weeks old mice were group-housed (four mice/cage; food and water ad libitum, room temperature 20-22°C) under a 12/12h light/dark cycle (light on at 7.30 am). Animals were purchased from Charles Rivers Laboratories (L'Arbresle Cedex, France). $\beta2^{-/-}$ mice were generated from a 129/Sv embryonic stem cell line as previously described (Picciotto et al. 1995) and backcrossed onto the B6 strain for 20 generations. All experiments were carried out to reduce the discomfort of mice in accordance with the EU Directive 2010/63/EU, Decree N 2013-118 of February 1st, 2013, and the French National Committee (87/848).

Experimental procedures (Figure 1)

All experiments were performed from 9.00 a.m. to 2.00 p.m. with 14-25 weeks old mice and conducted for both B6 and $\beta2^{-/-}$ mice.

After acclimatization to the experimental room, animals were either immediately sacrificed (Control mice, Cnt, n = 9 B6, n = 6 $\beta2^{-/-}$ mice), submitted to an acute stress (S mice), or submitted to acute stress or not and a social task (SESI-mice and ESI-mice). Stress was realized by placing the mouse for 45 minutes in a Falcon® tube opened at the end to permit breathing. Social task was realized by placing an Isolated Host mouse (IH, either stressed or not) and a Social Visitor mouse (SV, never stressed) together for the first time in a novel

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environment. The IH mouse, previously isolated for 3-4 weeks, was allowed to explore the novel environment for 30 min (E) before gently introducing an unfamiliar SV mouse in the box (always a B6 socially housed mouse). SV mouse was approximately of same weight and age than IH mouse. Social interactions (SI) between mice lasted 8 min. They were recorded for further analysis using Mice Profiler software (de Chaumont et al. 2012).

Mice submitted to stress alone were sacrificed 90 min after the beginning of stress (short delay protocol, S-Short, n = 6 B6, n = 7 $\beta2^{-/-}$ mice, published data, Nosjean et al. 2018) or 90 min after its end (long delay protocol, S-Long, n = 7 B6, n = 7 $\beta2^{-/-}$ mice, current data). Stressed mice subjected to exploration and social interaction were sacrificed 90 min after the beginning of stress (short delay protocol, SESI-Short, n = 8 B6, n = 8 $\beta2^{-/-}$ mice, published data, Nosjean et al. 2018) or 90 min after the end of the social interaction (long delay protocol, SESI-Long, n = 10 B6, n = 7 $\beta2^{-/-}$ mice, current data). Non-stressed mice were also sacrificed 90 min after the end of the social interaction (long delay protocol, ESI-Long, n = 8 B6 and n = 8 $\beta2^{-/-}$ mice, current data).

Immunohistochemical procedures and quantification

General immunohistochemical procedures and quantification were previously described in details (Nosjean et al. 2018). Briefly, after deep anesthesia (Dolethal 2ml / 3ml Nacl, i.p. 0.1ml / 10g) and intracardiac perfusion of fresh ice cold 4% paraformaldehyde in phosphate buffer saline (PBS), cryoprotected IH brains (20% sucrose in PBS overnight, 4°C°) were cut as serial free-floating coronal sections (40 µm) using a freezing microtome. Rabbit polyclonal anti-c-fos (c-fos (ab-5), 1:8000 dilution, Calbiochem #PC38), biotinylated goat anti-rabbit IgGs secondary antibody (1:200, Vector BA-1000), avidin-biotin complex (1:200 dilution,

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Vectastain Elite PK 6100) and diaminobenzidine tetrahydrochloride were sequentially used to reveal c-fos protein.

Brain images were acquired at x10 with a Sony DFW-X700 digital camera (Sony Co., Tokyo, Japan) coupled to Olympus BX60 light microscope (Olympus Optical Co., Hamburg, Germany). C-fos positive cells per square micrometer were quantified using *icy.bioimageanalysis.org* after delineating each region of interest (ROI) on the picture. C-fos positive cells were mapped in all brain structures in front of the corpus callosum from the frontal cortex (defined here for simplicity reasons as the prefrontal cortex, PFC, from bregma +3.08 to +1.54, Paxinos and Franklin 2001), and in the basolateral nucleus of the amygdala (BLA, from bregma -0.58 to -1.58 mm).

In the PFC, for structures extending over more than one rostro-caudal level (i.e., PrL, Cg1, M2, M1, LO, VO and MO, 7 areas), we pooled quantifications obtained from its rostral to its caudal extent (Global PFC). We also analyzed c-fos protein expression by level in the PFC, i.e., in the rostral (from bregma 3.08 to 2.58 mm), median (from bregma 2.46 to 2.10 mm), and caudal parts (from bregma 1.98 to 1.54 mm) of the PFC. For each mouse and each selected brain level, at least 2-3 sections were bilaterally analyzed (i.e., about more than 100 ROIs par mouse) and then averaged for each animal.

Statistical analysis

C-fos protein expression was quantified in the global PFC and in its 24 sub-areas as well as in the BLA of the amygdala. Results were expressed as means ± SEM. Values were considered statistically significant for $p \leq 0.05$. Experiments were analyzed in 2 sets. In the first one, comparisons were performed on 10 independent groups, i.e., for both genotypes, in control mice (no stress and no behavioral procedure), in stressed mice (S mice) and in stressed mice

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submitted to the social task (SESI mice) in which c-fos protein was detected either 90 min after the beginning of the experiment, i.e., after the beginning of the stress (S-Short and SESI-Short mice), or 90 min after the end of the experiment, i.e., after the end of the stress or the social interaction (S-Long and SESI-Long mice). In the second set of experiments, comparisons were performed on 4 independent groups, i.e., for both genotypes, in non-stress (ESI-Long) and stressed (SESI-Long) animals. Multiple comparisons between groups were performed with Kruskal-Wallis one-way ANOVA on Ranks using Sigmaplot 12.0. When normality passed, data were subsequently analyzed with the Holm-Sidak method test and when it did not, with Mann-Whitney U-tests. Correlations between the 25 studied brain areas were performed using Spearman test and a Bonferroni correction was applied for multiple comparisons. Statistically threshold was thus set at $p \leq 0.002$.

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Results

Effect of time, stress and social interaction on brain activity

In a first series of experiments, we dissected the time effect on brain c-fos protein expression in B6 and $\beta2^{-/-}$ mice subjected to acute stress alone or to acute stress followed by social interaction task (Fig. 1).

Effect of time on stress alone (Figure 2A-C, Supplementary Table 1a-b)

Acute stress in B6 mice **(S-Short vs Cnt)** induced a significant increase of c-fos protein expression in the **PrL** due to specific activation of the **rPrL,** in the **Cg1 (mCg1 and cCg1),** in the **M2** (mM2 and cM2), in **cM1** and in the **BLA**. Thus, at short time, acute stress induced brain activation all along the PFC, in particular in the median wall (**PrL, Cg1**), in the associative and motor areas (**FrA, M1, M2**) as well as in the **BLA**. In $\beta2^{-/-}$ mice, acute stress followed by a

short delay (**S-Short vs Cnt,** Nosjean et al. 2018) decreased c-fos protein expression only in **LO (rLO)**, **rVO, mPrL** and in the **BLA**. Thus, acute stress triggered opposite effects in B6 and $\beta2^{-/-}$ mice compared to their respective controls. Limbic structures such as **Prl, Cg1** and the caudal motor cortices (primary and secondary) and **BLA** were hyper-activated by stress in B6 mice but not in $\beta2^{-/-}$ mice while other PFC areas (i.e., **mPrL** and orbital **rLO** and **rVO** areas) and **BLA** were hypo-activated by stress in $\beta2^{-/-}$ mice.

Long delay protocol showed no change in PFC c-fos protein levels, both in stressed B6 and $\beta2^{-/-}$ mice compared to their respective controls **(S-Long vs Cnt)**, when considering the entire PFC or its different levels. In contrast, an increase and a decrease of c-fos protein expression remained detected in the **BLA** respectively in B6 and $\beta2^{-/-}$ mice (for both genotypes, H = 36.143, $df = 9$, $p < 0.001$; MW: $p = 0.001$). Thus, time normalized the stress-induced c-fos protein expression in the PFC in both genotypes, but was not sufficient to restore that of the BLA.

Finally, comparisons between short and long delay protocols **(S-Long vs S-Short)** showed marginal hypo-activations taking placed only at some levels of the rostral axis: a diminution of c-fos protein expression was observed in B6 **mCg1** area (H = 48.526, $df = 9$, $p < 0.001$; MW: $p = 0.022$) and in $\beta2^{-/-}$ **rFrA** (H = 57.449, $df = 9$, $p < 0.001$; MW: $p = 0.026$). No change was detected in B6 **BLA** (H = 36.143, $df = 9$, $p < 0.001$; MW: $p = 1.000$) while a decrease in $\beta2^{-/-}$ **BLA** was evidenced (H = 36.143, $df = 9$, $p < 0.001$; MW: $p = 0.007$). Such results suggest that time has little if any effect on stress-induced c-fos expression in the PFC, while it influenced the BLA activity, only in $\beta2^{-/-}$ mice. This genotype difference discards a simple and non-specific delay effect of cfos protein detection with time.

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Effect of time on stress followed by social task (Figure 2A-E, Supplementary Table 2a and b)

The influence of time was also assessed on social interaction in stressed animals (**SESI-Short vs Cnt, SESI-Long vs Cnt, SESI-Long vs SESI-Short**).

We observed that B6 mice submitted to acute stress followed by the social task (**SESI-Short vs Cnt**) showed increased c-fos protein expression in all prefrontal areas ($0.03 \leq ps < 0.001$) except in the **mVO,** the **mMO** and the **cLO** (Nosjean et al. 2018). Likewise, $\beta2^{-/-}$ animals displayed increased activity compared to their controls ($0.011 \leq ps < 0.001$), except in the **MO** when considering the PFC areas in all their extent and in the **rMO**, **rDLO**, **mAI**, **mMO, cAID, cAIV** and **cLO** along the PFC rostro-caudal axis (Nosjean et al. 2018). For both genotypes, hyperactivity was observed in the **BLA** (H = 36.143, $df = 9$, $ps < 0.001$; MW: $ps < 0.001$). Thus, even if social interaction occurs shortly before sacrifice, i.e., with little time for c-fos expression to change, it produced massive brain activation in stressed mice of both genotypes.

Long delay protocol induced no change in c-fos protein levels when looking at the entire PFC in stressed B6 mice submitted to the social task compared to their own controls **(SESI-Long vs Cnt)**. Along the rostro-caudal axis, it provoked hypo-activity in **rFrA** (H = 57.449, $df = 9$, $p < 0.001$; MW: $p = 0.002$) and over-activity in **mPrL** (H = 43.911, $df = 9$, $p < 0.001$; MW: $p = 0.037$), **mCg1** (H = 48.526, $df = 9$, $p < 0.001$; MW: $p = 0.045$) and **BLA** (H = 36.143, $df = 9$, $p < 0.001$; MW: $p = 0.030$). In contrast, in $\beta2^{-/-}$ mice **(SESI-Long vs Cnt)**, long delay triggered a drop of c-fos protein expression only in **LO** considering the PFC as a whole (H = 52.467, $df = 9$, $p < 0.001$; MW: $p = 0.035$) likely due to decreases in the **rLO** (H = 53.706, $df = 9$, $p < 0.001$; MW: $p = 0.005$) and **mLO** (H = 53.706, $df = 9$, $p < 0.001$; MW: $p = 0.014$). Other PFC areas also showed hypo-activity: **rFrA** (H = 57.499, $df = 9$, $p < 0.001$; MW: $p = 0.002$), **rVO** (H = 46.088, $df = 9$, $p < 0.001$; MW: $p = 0.005$), **mPrL** (H = 43.911, $df = 9$, $p <$

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0.001; MW: $p = 0.005$) and in **BLA** (H = 36.143, $df = 9$, $p < 0.001$; MW: $p = 0.001$). Thereby, adding a delay between the social interaction and the mice sacrifice drastically changed its impact on brain c-fos protein expression: it diminished the extent of the PFC activation in B6 mice and reversed its impact in $\beta2^{-/-}$ mice in which only hypo-activations were detected. Thus, time buffered the stress-social interaction induced c-fos protein activation.

In line with previous work (Nosjean et al. 2018) after a short delay **(SESI-Short vs S-Short)**, social task in stressed mice compared to stress alone triggered over-activations in all rostral areas and only in part in medial and caudal PFC areas (**mM1**, **cM1, rMO, mPrL**, **cSI** and **cDI)** in B6 mice while in $\beta2^{-/-}$ mice only over-activations were detected in the **PFC (**except in the **cLO)** and in the **BLA.** Thus, social task in stressed mice induced hyper-activation at short term, in particular in $\beta2^{-/-}$ mice.

After long delay **(SESI-Long vs S-Long)**, social behavior in stressed mice induced no statistical effect for any analyzed PFC area in B6 and $\beta2^{-/-}$ mice apart from a hypo-activation in **rFrA** for both genotypes (H = 57.449, $df = 9$, $p < 0.001$; MW: $p = 0.002$ in B6 mice, $p = 0.038$ in $\beta2^{-/-}$ mice). In the **BLA**, c-fos protein level dropped in B6 (H = 36.143, $df = 9$, $p < 0.001$; MW: $p = 0.002$) but remained unchanged in $\beta2^{-/-}$ mice (H = 36.143, $df = 9$, $p < 0.001$; MW: $p = 0.535$). Thus, the long delay that allowed a maximal expression of c-fos protein revealed a strong buffering role of social interactions on stress-induced prefrontal activation.

Finally, the long delay protocol **(SESI-Long vs SESI-Short)** provoked hypo-activations in all brain studied areas in both in B6 and $\beta2^{-/-}$ mice, except for B6 mice in **mVO** ($F_{(9,65)} = 8.243$, $p < 0.001$; HS: $p = 0.614$), **mMO** ($F_{(9,65)} = 6.193$, $p < 0.001$; HS: $p = 0.294$) and **cLO** ($F_{(9,65)} = 3.414$, $p < 0.001$; HS: $p = 0.874$). Hence, despite some exceptions, the delay before sacrifice induced hypo-activations in the PFC and in the BLA in both genotypes.

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Genotype and time effects (Figure 3A-B, Supplementary Table 4a-b)

Comparisons between B6 and β2$^{-/-}$ mice (**β2$^{-/-}$ vs B6 mice**) were done for control mice (**Cnt**), stress alone with a short (**S-Short**) or a long (**S-Long**) delay of c-fos protein expression, and stress followed by the social task with a short (**SESI-Short**) or long delay (**SESI-Long**).

No statistical differences for any analyzed area were detected between B6 and β2$^{-/-}$ control mice when considering the PFC or the BLA.

With the short delay following stress alone significant decreases of the protein expression were observed in all PFC areas taken in all their extent in β2$^{-/-}$ mice compared to B6 mice except in **MO** (**S-Short,** $0.029 \leq ps < 0.001$). Hypo-activations were specifically due to diminutions of the protein expression at different levels of the rostro-caudal axis, i.e., in the **rLO**, **rVO, mPr**L **cPrL mCg1, cCg1, mM2, cM2** and in the **BLA** which is in concordance with previous data (Nosjean et al. 2018). Hence, in β2$^{-/-}$ mice, a short delay mostly reduced the protein expression in the orbital structures of the rostral PFC as well as in the medio-caudal wall of the PFC, and in the amygdala, suggesting an important role of β2-nAChRs in these areas.

Stress followed by a long delay in β2$^{-/-}$ mice compared to B6 mice **(S-Long)** significantly decreased the activity of **PrL** ($F_{(9,65)} = 16.553$, $p < 0.001$; HS: $p = 0.049$) and **Cg1** ($F_{(9,65)} = 15.582$, $p < 0.001$; HS: $p = 0.048$), likely because of effect on the **rPrL** ($F_{(9,65)} = 15.010$, $p < 0.001$; HS: $p = 0.006$) and on the **cCg1** ($F_{(9,65)} = 14.671$, $p < 0.001$; HS: $p = 0.041$) as other regions remained unaffected. Diminutions in the rostral PFC were also detected, specifically in the **rFrA** ($H = 57.449$, $df = 9$, $p < 0.001$; MW: $p = 0.038$) and in the **rLO** ($H = 53.706$, $df = 9$, $p < 0.001$; MW: $p = 0.038$). In addition, **BLA** was hypo-activated ($H = 36.143$, $df = 9$, $p < 0.001$; MW: $p < 0.001$). Thus, long delay significantly extended hypo-activation in the rostral PFC and in the BLA of β2$^{-/-}$ mice.

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Short delay protocol in stressed β2$^{-/-}$ mice interacting socially compared to their corresponding B6 mice **(SESI-Short)** didn't show any **BLA** (H = 36.143, *df* = *9*, *p* < 0.001; MW: *p* = 0.574) nor **PFC** alterations except in **rFrA** (H = 57.449, *df* = *9*, *p* < 0.001; MW: *p* < 0.001). Thereby, the major genotype effect in stressed β2$^{-/-}$ mice submitted to social task is a restoration of c-fos protein expression in the PFC and in the BLA.

In contrast, at long delay after stress and social task **(SESI-Long)** there was significant hypo-activations in β2$^{-/-}$ mice compared to B6 mice in the **PrL** ($F_{(9,65)}$ = 16.553, *p* < 0.001; HS*:* *p* = 0.013), **Cg1** ($F_{(9,65)}$ = 15.582, *p* < 0.001; HS*:* *p* = 0.001), **LO** (H = 52.467, *df* = *9*, *p* < 0.001; MW: *p* < 0.001) and **BLA** (H = 36.143, *df* = *9*, *p* < 0.001; MW: *p* < 0.001). Looking at the different levels of the PFC, decreases of the protein expression were significant in the **rFrA** (H = 57.449, *df* = *9*, *p* < 0.001; MW: *p* = 0.017), **rLO** (H = 53.706, *df* = *9*, *p* < 0.001; MW: *p* = 0.003), **rVO** ($F_{(9,65)}$ = 46.088, *p* < 0.001; HS*:* *p* < 0.001), in the **mPrL** (H = 43.911, *df* = *9*, *p* < 0.001; MW: *p*= 0.002), **mCg1** (H = 48.526, *df* = *9*, *p* < 0.001; MW: *p*= 0.002), **mM2** (H = 45.939, *df* = *9*, *p* < 0.001; MW: *p* = 0.028), **mLO** (H = 40.675, *df* = *9*, *p* < 0.001; MW: *p*= 0.001) and the **cIL** ($F_{(9,65)}$ = 10.503, *p* < 0.001; HS*:* *p* = 0.002), **cPrL** ($F_{(9,65)}$ = 11.498, *p* < 0.001; HS*:* *p* = 0.016) and in the **cCg1** ($F_{(9,65)}$ = 14.671, *p* < 0.001; HS*:* *p* = 0.001). Hence, long delay amplified hypo-activation all along the PFC and in the BLA in β2$^{-/-}$ mice. As this was not the case in B6 mice, we can rule out a general timing effect of c-fos protein detection. Notably, the genotype effect triggered by any behavioral manipulation (stress, social behavior, at short or long delay) was mainly reflected by hypo-activation of the PFC and of the BLA, but never by hyper-activations.

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Brain functional connectivity (Figure 4A-B)

Statistical correlations were calculated to identify putative functional relationships between the different PFC areas and the BLA, i.e., between 25 areas.

In B6 control mice, 17 positive correlations were detected between PFC sub-areas ($0.00199 \leq ps \leq 0.0000002$), mainly between lateral and medial areas all along the rostro-caudal axis. Stress alone totally disconnected PFC sub-areas activity in B6 mice at short delay **(S-Short)** but triggered numerous PFC relationships at long delay (41 correlations, **S-Long,** *ps* = 0.0000002). These functional connections were evidenced within each level of the PFC as well as between the lateral, medial and latero-medial areas. Only two PFC sub-areas (**mVO and cCg1**) established functional connectivity with the BLA. B6 mice having a social interaction after stress showed additional correlations at short delay (**SESI-Short**, 8 correlations; *ps* = 0.0000002) but drastically disconnected PFC sub-areas at long delay (**SESI-Long**, 6 correlations; *ps* = 0.0000002). Only **cPrL** and **cIL** remained steadily correlated whatever the delay, when social interaction followed stress.

No significant correlations were found in $\beta2^{-/-}$ control animals while stress alone triggered some correlations between PFC areas after short delay, mostly between medial PFC areas (**S-Short**, 11 positive and 1 negative (bold arrow, *ps* = 0.0000002). After long delay, stress massively extended functional connectivity within the PFC of $\beta2^{-/-}$ mice (**S-Long**, 82 significant correlations, *ps* = 0.0000002). Compared to stress alone, correlations following social interaction in stressed $\beta2^{-/-}$ animals increased after short delay (**SESI-Short**, 21 correlations, $0.00178 \leq ps \leq 0.0000002$), and drastically diminished after long delay (**SESI-Long**, 17 correlations, *ps* = 0.0000002). Only **cCg1** and **cPrL** remained steadily correlated whatever the delay, when social interaction followed stress in $\beta2^{-/-}$ mice. With time, a very limited number

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of correlations were similar in B6 and β2$^{-/-}$ mice after stress (**S-Long**, 11 correlations) and after stress coupled to social task (**SESI-Long**, 2 correlations, Fig. 4A and B, dotted arrows).

Overall, whatever the experimental procedure, major correlations within PFC sub-areas markedly differed between B6 and β2$^{-/-}$ mice, while the BLA activity remained mostly independent of the PFC activity. With the long delay stress alone drastically triggered plethora of functional interconnections between PFC sub-areas, while having a social interaction drastically disconnected them in both genotypes. Thus, major changes in the functional connectivity and organization of cortical networks were detected after extended delay.

Does stress affect brain activity after a long delay (Figures 1, 5A-B, 6, Supplementary Tables 5a-b)?

In a second series of experiments, we also evaluated the impact of time in stressed and non-stressed mice (**SESI-Long and ESI-Long**, respectively).

With time, stress triggered hypo-activations in all **PFC** areas when considering the entire PFC or its different levels both in B6 and in β2$^{-/-}$ mice interacting socially ($0.022 \leq ps < 0.001$ for B6 mice and $ps \leq 0.001$ for β2$^{-/-}$ mice) and in **BLA,** specifically in β2$^{-/-}$ mice ($F_{(3,32)} = 31.413$, $p < 0.001$; HS: $p = 0.002$). Indeed, stress had no effect on BLA in B6 mice, suggesting a different emotional value of stress in the two genotypes.

We also assessed the genotype effect in stressed and non-stressed mice interacting socially for a long delay (**β2$^{-/-}$ vs B6 mice**). In non-stress condition, when looking at the entire PFC, brain activation didn't differ between the two genotypes except for significant increased activity in **M1** ($F_{(3,32)} = 34.044$, $p < 0.001$; HS: $p = 0.006$) and decreased activity in the **BLA** ($F_{(3,32)} = 31.413$, $p < 0.001$; HS: $p < 0.001$) in β2$^{-/-}$ mice. Looking at the different levels of the

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PFC, increases of the protein were detected in the median and caudal PFC in $\beta2^{-/-}$ mice. Such hyper-activations concerned **mCg1** (H = 26.937, *df* = *3*, *p* < 0.001; MW: *p* = 0.028), **mM1** (H = 24.451, *df* = *3*, *p* < 0.001; MW*: p* = 0.010), **mAI** ($F_{(3,32)}$ = 39.367, *p* < 0.001; HS*: p* = 0.028), **cM2** (H = 26.504, *df* = *3*, *p* < 0.001; MW: *p* = 0.050), **cM1** ($F_{(3,32)}$ = 37.173, *p* < 0.001; HS*: p* = 0.012), **cS1** ($F_{(3,32)}$ = 20.380, *p* < 0.001; HS*: p* = 0.007), **cAIV** (H = 24.764, *df* = *3*, *p* < 0.001; MW: *p* = 0.015) and **cLO** (H = 27.992, *df* = *3*, *p* < 0.001; MW: *p* = 0.015). Hence, in the non-stress condition the absence of functional β2-nAChRs increased activity in the PFC and decreased it in the BLA. Such over-expressions were especially seen in the median and caudal PFC, mostly laterally.

In the stress condition only decreases in activity were detected in $\beta2^{-/-}$ mice compared to B6 mice. They manifested in all PFC areas of interest, except in **M1** ($F_{(3,32)}$ = 34.044, *p* < 0.001; HS*: p* = 0.182), as well as in the **BLA** ($F_{(3,32)}$ = 31.413, *p* < 0.001; HS*: p* < 0.001). Significant hypo-activity in the global orbital areas were associated to decreases at each level of the **LO** (**rLO**: H = 25.706, *df* = *3*, *p* < 0.001; MW: *p* = 0.003; **mLO**: $F_{(3,32)}$ = 66.234, *p* < 0.001; HS*: p* < 0.001), **cLO**: H = 27.992, *df* = *3*, *p* < 0.001; MW: *p* = 0.005), **VO (rVO**: H = 26.960, *df* = *3*, *p* < 0.001; MW: *p* < 0.001; **mVO**: H = 26.864, *df* = *3*, *p* < 0.001; MW: *p* = 0.002) and **MO** (**rMO**: $F_{(3,32)}$ = 36.986, *p* < 0.001; HS*: p* = 0.015; **mMO**: $F_{(3,32)}$ = 47.799, *p* < 0.001; HS*: p* < 0.001). Likewise, hypo-activity in **Prl, Cg1** and in the motor cortices **M2** was due to diminutions in their medial and caudal parts **(mPrL**: H = 26.487, *df* = *3*, *p* < 0.001; MW: *p* = 0.002; **mCg1**: H = 26.937, *df* = *3*, *p* < 0.001; MW: *p* = 0.002; **cPrL**: $F_{(3,32)}$ = 38.701, *p* < 0.001; HS*: p* < 0.001; **cCg1**: H = 26.937, *df* = *3*, *p* < 0.001; MW: *p* = 0.002; **mM2**: $F_{(3,32)}$ = 31.219, *p* < 0.001; HS*: p* = 0.013; **cM2**: H = 26.504, *df* = *3*, *p* < 0.001; MW: *p* = 0.004). Decreased activity in the insular cortices also extended along the rostro-caudal axis (**mAI**: $F_{(3,32)}$ = 39.367, *p* < 0.001; HS*: p* = 0.004; **cAID**: $F_{(3,32)}$ = 22.630, *p* < 0.001; HS*: p* = 0.012 and **cAIV**: HS = 24.764, *df* = *3*, *p* < 0.001; MW: *p* = 0.013). Finally, decreases in c-fos protein expression were

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significant only in **cM1** ($F_{(3,32)} = 37.173$, $p < 0.001$; HS: $p = 0.018$). Therefore, as compared to baseline levels, the coupling of stress and social interaction relies on functional β2-nAChRs, specifically regarding the medial wall of the PFC and the BLA, in which significant hypo-activations emerged.

We also identified statistical correlations between the different PFC areas taken by levels and the BLA.

In non-stress B6 mice (**ESI-Long**), 14 positive correlations were detected between PFC sub-areas, mainly between lateral ones along the rostro-caudal axis ($0.00178 \leq ps \leq 0.0000002$). Stress drastically reduced such functional relationships (**SESI-Long**, 6 correlations, $0.0000002 \leq ps \leq 0.00178$). In non-stress $\beta2^{-/-}$ mice (**ESI-Long**), significant correlations were mainly reported in the latero-medial areas at each level of the rostro-caudal axis (19 correlations, $0.00178 \leq ps \leq 0.0000002$). Correlations were totally different from those identified in B6 mice, except relationships between mM1-cS1 and cCg1-cPrL areas. After stress (**SESI-Long**, 17 correlations, $ps = 0.0000002$), correlations between lateral and latero-medial areas were reduced, while those between medial areas appeared.

Overall, at long term the coupling of stress and social interaction drastically changed functional connectivity within the PFC. Although abundant in all brain areas, β2-nAChRs appear particularly important for functional connectivity within prefrontal sub-areas, and for its plasticity and reorganization after stress.

Discussion

The effects of stress exposure depend on many factors such as nature, intensity, duration of the stressor, age, sex and history of the individual. Behavioral and physiological adjustments to stress engage different systems as the neuroendocrine, autonomic, immune, cardiovascular and

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metabolic systems as well as brain release of different neurotransmitters, neuromodulators and neuropeptides (see Westerink 1995; McEwen 2006; Radley et al. 2008; Arnsten 2009; Lupien et al. 2009; Ossewaarde et al. 2011; McEwen et al. 2016), and many brain structures such as the hypothalamus, the hippocampus, the ventral tegmental area, the nucleus accumbens, the PFC and the amygdala, among others (see Cacioppo 1994; Herman and Cullinan 1997; Buijs and Van Eden 2000; Pacák and Palkovits 2001; de Kloet et al. 2005; Arnsten 2009; Lupien et al. 2009; Mora et al. 2012; Beery and Kaufer 2015; McKlveen et al. 2015; Paul et al. 2015; Mc Ewen et al. 2016; Zhang et al. 2018). Brain c-fos protein expression depends on the intensity and the duration of the stimulus and on its acute or chronic character. In the literature acute restraint stress duration has been reported to be either of 30 min (Yokoyama and Sasaki 1999; Chowdhury et al. 2000; Viau and Sawchenko 2002; Patel et al. 2005; Radley et al. 2008; Goebel et al. 2009; Zavala et al. 2011), 60 min (Stone et al. 1993; Yokoyama and Sasaki 1999; Chowdhury et al. 2000), two hours (Honkaniemi et al. 1992; Chowdhury et al. 2000; O'Mahony et al. 2010) or more (2 at 8 hours; Honkaniemi et al. 1992; Chowdhury et al. 2000; Roske et al. 2002). The period between the end of the stimulus and the sacrifice of animals for c-fos protein detection also differs: for various stress durations, the delay before sacrifice can be just after stress exposure (Yokohama et al. 1999; Chowdhury et al. 2000; Viau and Sawchenko 2002; Patel et al. 2005; Reznikov et al. 2008; Ito et al. 2009), of 30 min (Yokohama et al. 1999; Viau and Sawchenko 2002; Patel et al. 2005), of 1 hour (Yokohama et al. 1999; Viau and Sawchenko 2002), of 2 hours (Stone et al. 1993; Radley et al. 2006, 2008; O'Mahony et al. 2010; Zavala et al. 2011), or of several hours (Chowdhury et al. 2000; Viau and Sawchenko 2002). Here, with an acute and fix stress duration of 45 min, we addressed the question of impact on brain activity of 1) a time laps between stress coupled or not with a social task, and sacrifice 2) a long delay between social interaction and sacrifice, in stress and no stress conditions, in order to address the plasticity of PFC-BLA networks.

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Effect of time on brain plasticity versus time-course of c-fos protein expression.

We could wonder whether using c-fos is a suitable method to capture the effect of time and its buffering role, as c-fos protein expression varies with time and, as described above, decreases with time. If our results were only due to the time-course of the c-fos expression, we could expect brain activation and brain connectivity to reach control levels after a certain time. However, our results demonstrate that between short and long delays, we observed either no effect, hyper-activation or hypo-activation, depending on the behavior and of the lack of β2 nAChRs. Similarly, when considering functional connectivity, there was no linear modification with time, thus discarding a simple effect of time-course of the protein expression.

Effect of time, stress, social interaction on brain activity.

First, we analyzed PFC and BLA c-fos protein expression at two time points, i.e., 90 min after the beginning or the end of stress exposure, coupled or not to a social task. Such timing was chosen in accordance with works showing a peak production of the protein between 60-120 minutes (Morgan et al. 1987; Morgan and Curran 1989; Hoffman et al. 1993; Kovacs 1998, 2008; Zangenehpour and Chaudhuri 2002; Guzowski et al. 2005). We chose to focus on the PFC and on the amygdala because of their key role in mediating the emotional responses to stress and social behavior outcomes (Heidbreder and Groenewegen 2003; McEwen et al. 2006, 2008; Lupien et al. 2009; Arnsten 2009; Avale et al. 2011; Mora et al. 2012; Nosjean et al. 2018). Furthermore, we questioned the role of the cholinergic system using mice lacking nicotinic β2-receptor subunit (β2-nAChRs), since brain cholinergic transmission was shown to play a major role in stress induced affective and behavioral responses (Robbins and Roberts 2007; Ofek and Soreq 2013; Mineur and Picciotto 2019; Neves and Grace 2019). The β2-

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nAChRs are ubiquitously expressed in the brain, notably in the PFC and the BLA (Hogg et al. 2003; Fowler et al. 2008; Hurst et al. 2013; Mineur et al. 2016; Zhang et al. 2018; Mineur and Picciotto 2019) and we previously showed that their viral re-expression specifically in the prelimbic area of the PFC was necessary and sufficient to restore social interaction in $\beta2^{-/-}$ mice (Avale et al. 2011).

Effect of time on stress alone

At short-term, stress impacted differentially the PFC and the BLA in both genotypes compared to their own control: it activated the BLA and some PFC areas all along the rostro-caudal axis in B6 mice, and hypo-activated the BLA and some other PFC sub-regions in $\beta2^{-/-}$ mice. Thus, the BLA and the PFC reacted in opposite way depending on the presence of the β2 nAChRs that play a buffer role in such stress processes. At long term, stress also impacted distinctly each genotype's PFC and BLA reactivity. Indeed, compared to controls, time fully buffers the stress-induced PFC hyper-activation in B6 mice but not the PFC hypo-activation in $\beta2^{-/-}$ mice. It had no effect on BLA activity in B6 mice and reinforced the BLA hypoactivation in $\beta2^{-/-}$ mice, suggesting that the cholinergic system plays a role on PFC and promotes BLA reactivity. Interestingly, comparison between short and long delay after stress showed little or no effects in the PFC and the BLA. Thus, the stress impacts fade with time in all PFC areas but was maintained in the BLA. Both stress and time differentially modulate the PFC and BLA activities depending on the presence of β2 nAChRs (Fig. 7).

Despite strong reciprocal anatomical connections between PFC sub-regions (Van Eden and Buijs 2000; Gabbott et al. 2003; Heidbreder and Groenewegen 2003; Dalgleish 2004; Vertes 2004, 2006; Rogers-Carter et al. 2018), stress in B6 mice completely deconstructs all functional links between PFC sub-areas at short time and triggers multiple PFC relationships at

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long term suggesting that functional connectivity is an important marker of stress adaptation that should be taken into account in brain imaging studies. By contrast, in β2$^{-/-}$ mice stress triggers some functional connectivity at short term and plethora at long term. These data evidence that both stress and time participate in network reconfiguration, likely involving β2 nAChRs modulation in the PFC. Analyzing correlation of activity between brain regions using c-fos expression provides an index of functional connectivity (Lesburguères et al. 2011 ; Cohen et al. 2017; Rogers-Carter et al. 2018), particularly as we quantified here c-fos expression in numerous prefrontal regions of interest shown to be part of an important social network (Rogers-Carter et al. 2018).

Frontal areas established dense reciprocal connections with the BLA (McDonald 1991, 1998; Bacon et al. 1996; Gray 1999; Vertes 2004; Gabbott et al. 2005; Hoover and Vertes 2007). However, like observed by others in social conditions (Rogers-Carter et al. 2018), we didn't observe functional relationships between the BLA and the PFC sub-regions. Such functional connection significantly emerged only when stress was followed by a long delay, and only in B6 mice, suggesting a role of β2 nAChRs in this PFC-BLA plasticity. Other authors reported that despite anatomical connections some areas (OFC, PrL, hippocampus) may not be activated together, depending on the behavioral contexts (Lesburguères et al. 2011; Rogers-Carter et al. 2018; Liu et al. 2020). Therefore, we can hypothesize that BLA and PFC may predominantly not act in synergy, except in stress condition after long delay. This synergy could be supported by the fact that stress promotes the release of monoamines, glutamate and GABA both in the PFC and in the amygdala (Imperato et al. 1991; Tanaka et al. 1991; Arnsten 1997; Finlay and Zigmond 1997; Feenstra 2000; Morilak et al. 2005; Moghaddam and Homayoun 2008; see also Arnsten and Li 2005; Mora et al. 2012). Acetylcholine, however, plays a distinct role in stress effect. Indeed, stress promotes the release of Ach in the PFC (Mark et al. 1996;

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Pepeu and Blandina 1998; Mora et al. 2012; Ofek and Soreq 2013) but not in the amygdala (Mark et al. 1996). We previously showed that β2$^{-/-}$ mice exhibit higher levels of monoamines and of Ach than B6 mice in the prelimbic area (Coura et al. 2013), and show altered PFC excitatory/inhibitory balance (Pittaras et al. 2016). The differential impact in both strains on stress-induced brain activity/plasticity could be linked to differences in levels of various neurotransmitters, leading to distinct PFC-BLA reactivity to stress.

Effect of time on stress followed by social task

The strong brain c-fos over-expression associated to social interaction occurring in our case 30 minutes after stress in our short-term condition is independent of the time necessary for the maximal protein translation. Brain activation was also seen by others when protein expression was measured just after the end of a stressful experiment (Chowdhury et al. 2000; Viau and Sawchenko 2002; Patel et al. 2005; Reznikov et al. 2008; Ito et al. 2009). Moreover, such effects on PFC and BLA activity are specifically due to the social interaction since novelty exploration without social behavior after stress induced only marginal activation of the PFC in B6 mice and no activation in β2$^{-/-}$ mice (Nosjean et al. 2018). When increasing the delay after social interaction the extent of the PFC hyper-activation was nearly abolished, thus reaching normal levels in B6 mice. PFC activation was, by contrast, completely reversed in β2$^{-/-}$ mice, in which only hypo-activations were detected. Likewise, only hypo-activations were detected in the entire PFC and in the BLA in both genotypes when comparisons were performed between short and long delays, or in mice submitted to the social task without stress (see Fig. 7). Our data therefore indicates that time alone is not sufficient to restore normal brain activity and that the opportunity to have a social contact after stress promotes brain plasticity.

Stress followed by social task after a long delay differentially engaged PFC and BLA activity in all mice. This is in accordance with the literature assigning a major role of the PFC

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in cognitive and executive processes and the one of amygdala in affective and avoidance behaviors (see Mc Ewen 2007; del Arco and Mora 2009; Higley and Picciotto 2014; Neves and Grace 2019). We previously showed that acute stress triggers aggressiveness in B6 and $\beta2^{-/-}$ mice, even if not all individuals were affected (Nosjean et al. 2015). After a short delay, social interaction restores a few functional links between PFC areas in stressed B6 mice and increases functional connectivity in stressed $\beta2^{-/-}$ mice. Increasing the delay after stress coupled to social task drastically reduced the number of functional relationships in both genotypes. Taking together, our data show that functional PFC-BLA connectivity is not only modulated by behavior but also by time and β2 nAChRs activity. Social interaction, known to involve the cholinergic system, and time may together synergistically down-regulate the impact of acute stress and participate in resilience modulation, thus extending the works reviewed by Beery and Kaufer (2015).

Does stress affect brain activity after a long delay?

With time, stress coupled with social task hypo-activated all PFC areas in both genotypes while in non-stress condition, restoration to basal level requires β2-nAChRs. Likewise, β2-nAChRs appeared very important for the BLA activity, whatever the emotional state (Fig. 7). Moreover, at long term, coupling acute stress and social task drastically changed the functional connectivity within the PFC but had no effect on the BLA. Thus, β2-nAChRs appear particularly important for functional connectivity within prefrontal sub-areas, and for its plasticity and reorganization after stress.

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Conclusions

Our main results reveal that time fully buffers the stress-induced PFC and BLA hyper-activation in B6 mice. The lack of β2-nAChRs provoked massive PFC and BLA hypo-activations and modifies the functional connectome within the PFC and the functional PFC-BLA connectivity. Part of the PFC areas studied as the dorsomedial PFC, the rostral anterior cingulate cortex, the anterior and ventral median PFC, the rostral insula and the sensory motor areas are largely involved in the default mode network (DMN) characterized by a deactivation of functional brain activity during various tasks and an activation at rest (see Raichle et al. 2001; Pallesen et al. 2009; Raichle 2015; Mooneyham et al. 2016; Clemens et al. 2017; Finc et al. 2017). As illustrated in Fig. 6, our social task following stress abolished such functional connectivity in the PFC-BLA network. If further works are needed to fully understand the relationship between brain activity and functional connectivity our results clearly show a time dependent brain plasticity.

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Notes

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Captions to figures

Fig. 1. Experimental procedures. Isolated host B6 or β2$^{-/-}$ mice (IH) and B6 social visitor mice (SV) were kept in experimental room for 1 hour after their transfer from animal facility. Then, not stressed mice were immediately sacrificed for brain c-fos protein detection (control mice, **Cnt**). Mice submitted to stress alone (**S**) or stress and social task (**SESI**, i.e., stress (S) followed by exploration (E) and social interaction (SI)) were sacrificed either 90 min after the beginning of the stress procedure (Short delay, solid lines) or 90 min after the end of the stress or of the social interaction (Long delay, dotted lines) to allow an identical induction time of c-fos protein between S and SESI groups. **ESI** mice, i.e., not stress mice submitted to exploration and social interaction were sacrificed 90 min after the end of the social interaction (Long delay, dotted lines). IH mouse submitted to the social task was placed in the experimental box for a 30 min novelty exploration period (E) before to introduce a SV mouse in the box during 8 min to study mice social interactions (SI).

Fig. 2. Stress, social task and time effects on c-fos protein expression. A-B: Quantification of c-fos protein in the PFC and in the BLA in B6 **(A)** and β2$^{-/-}$ **(B)** mice subjected to no stress and no behavior (**Cnt**), to acute stress alone (**S mice**) and to acute stress followed by social task (**SESI mice**). Mice were sacrificed either 90 min after the beginning of the stress (**S-Short** and **SESI-Short**) or 90 min after the end of the stress or of the social interaction (**S-Long** and **SESI-Long**). Histograms are means ± SEM. Significant differences between groups are reported between bars ($0.043 \leq ps < 0.001$ for B6 mice, $0.038 \leq ps < 0.001$ for β2$^{-/-}$ mice). PFC and amygdalar sub-areas were distinguished using the atlas of Paxinos and Franklin (2001). Cortical areas were considered either in their extent (from bregma +3.08 to +1.54 mm, Global PFC) or in relation to their rostral (from bregma 3.08 to 2.58 mm, Rostral PFC), median (from bregma 2.46 to 2.10 mm, Median PFC) and caudal (from bregma 1.98 to 1.54 mm, Caudal PFC) location. Letters (r, m, c) preceding the structure's name indicate respectively their rostral,

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median or caudal level. The BLA was considered as extending from bregma -0.58 to -1.58 mm. AI: agranular insular cortex; AID: agranular insular cortex, dorsal part; AIV: agranular insular cortex, ventral part; BLA: basolateral amygdaloid nucleus, anterior part; Cg1: cingulate cortex, area 1; DI: dysgranular insular cortex; DLO: dorsolateral orbital cortex; FrA: frontal association cortex; IL: infralimbic cortex; LO: lateral orbital cortex; M1: primary motor cortex; M2: secondary motor cortex; MO: medial orbital cortex; PrL: prelimbic cortex; S1: primary somatosensory cortex; VO: ventral orbital cortex. **C-E:** Schematic illustrations of c-fos protein immunoreactivity in representative coronal sections of the rostral, median and caudal PFC in B6 and $\beta2^{-/-}$ mice. Significant increases or decreases of protein expression are indicated by respectively dark and light gray areas (see supplementary Tables 1a and b, 2a and b, 3a and b, for significance of the results). Distance to bregma is pointed on the right of the coronal sections. B6: Cnt, n = 9; S-Short, n = 6; S-Long, n = 7; SESI-Short, n = 8; SESI-Long, n = 10. $\beta2^{-/-}$: Cnt, n = 6; S-Short, n = 7; S-Long, n = 7; SESI-Short, n = 8; SESI-Long, n = 7.

Fig. 3. Genotype effects. $\beta2^{-/-}$ mice were compared to B6 mice for each experimental procedure, i.e., in control mice (**Cnt**), after a short and a long delay of c-fos protein expression in mice subjected to stress alone (**S-Short** and **S-Long**) and to stress followed by social task (**SESI-Short** and **SESI-Long**). Quantification **(A)** and schematic summary **(B)** of c-fos protein expression in the PFC and in the BLA. Significant differences are indicated ($0.029 \leq ps \leq 0.001$, see supplementary Tables 4a and b for significance of the results). In the representative coronal sections of the rostral, median and caudal PFC and of the amygdala, light gray areas indicate significant decreases of protein expression. Distance to bregma is reported on the right of the coronal sections (Paxinos and Franklin, 2001). See Fig. 2 for detailed information on brain subdivisions and brain structures abbreviations, and the number of mice used for each group.

Fig. 4. Correlations between PFC sub-areas and the BLA in B6 (A) and $\beta2^{-/-}$ mice (B) submitted to different experimental procedures, i.e., in control mice (**Cnt**), after short and long

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delay of c-fos protein expression in mice submitted to stress alone (**S-Short** and **S-Long**) and to stress and social task (**SESI-Short** and **SESI-Long**). Solid arrows indicate positive correlations, bold arrow negative ones. Significant differences following Bonferroni correction are indicated ($0.0000002 \leq ps \leq 0.00178$ for B6 mice, $ps = 0.0000002$ for $\beta2^{-/-}$ mice). Dotted arrows indicate correlations common to both genotypes for S-Long and SESI-Long protocols. See Fig. 2 for detailed information on brain subdivisions and brain structures abbreviations, and the number of mice used for each group.

Fig. 5. Stress and social task effects on long term c-fos protein expression in B6 and $\beta2^{-/-}$ mice. Quantification **(A)** and schematic illustration **(B)** of protein expression in the PFC and in the amygdala in stressed and not stressed B6 and $\beta2^{-/-}$ mice submitted to the social task and sacrificed 90 min after the end of the social interaction (**SESI-Long** and **ESI-Long**, respectively). Histograms are means ± SEM. Significant differences are indicated for each genotype between stressed and not stressed mice ($0.002 \leq ps < 0.001$ for B6 mice, $ps \leq 0.001$ for $\beta2^{-/-}$ mice), and between genotype for not stressed ($0.028 \leq ps < 0.006$ for ESI-Long mice) and stressed mice ($0.012 \leq ps < 0.001$ for SESI-Long, see supplementary Table 5a and b for significance of the results). In the representative coronal sections of the rostral, median and caudal PFC and of the BLA, light gray areas indicate significant decreases of protein expression and dark gray areas indicate significant increases. Distances to bregma are indicated on the right of the coronal sections (Paxinos and Franklin, 2001). See Fig. 2 for detailed information on brain subdivisions and brain structures abbreviations. ESI-Long mice: n = 8 for both genotypes, SESI-Long mice: n = 10 B6 and n = 7 $\beta2^{-/-}$ mice.

Fig. 6. Correlations between PFC sub-areas and the amygdala in B6 (A) and $\beta2^{-/-}$ mice (B) submitted or not to acute stress and to the social task. Mice were sacrificed 90 min after the end of social interaction (**SESI-Long** and **ESI-Long**, respectively). All correlations (arrows)

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are positive (statistical thresholds after Bonferroni correction: $0.0000002 \leq ps \leq 0.00178$ for B6 mice, $ps = 0.0000002$ for $\beta2^{-/-}$ mice). Dotted arrows indicate correlations common to both genotypes for S-Long and SESI-Long protocols. See Fig. 2 for detailed information on brain subdivisions and brain structures abbreviations. B6: ESI-Long, n = 8; SESI-Long, n = 10. $\beta2^{-/-}$: ESI-Long, n = 8; SESI-Long, n = 7.

Fig. 7. Schematic representation of the temporal evolution of c-fos protein expression depending on time, behavior and emotional status (stress or not) in the PFC (left) and in the BLA (right) in B6 and $\beta2^{-/-}$ mice (solid and dotted lines, respectively). C-fos protein was detected 90 (short) and 180 min (Long) after stress alone and after stress coupled with the social task, and 180 min after the social task without stress. Note that in the PFC, both genotypes exhibited similar profiles with a peak of activity when mice were submitted to stress coupled with the social task and sacrificed 90 min after the beginning of stress or when mice, submitted to the social task without stress were sacificed 180 min after the end of the social interaction. By contrast, temporal profile of protein expression in the BLA totally differs between genotypes.

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Figure 1

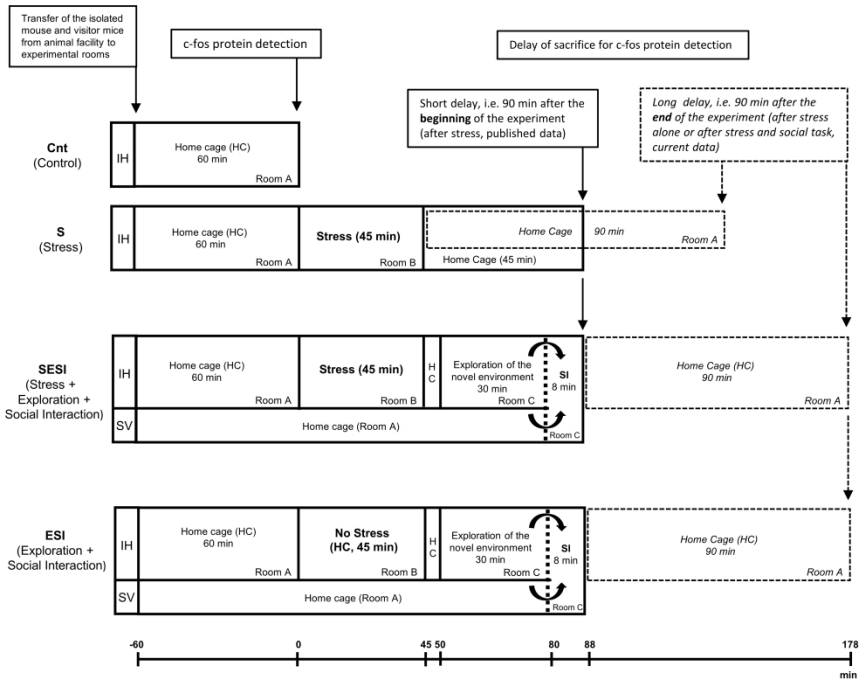

Fig. 1. Experimental procedures. Isolated host B6 or β2-/- mice (IH) and B6 social visitor mice (SV) were kept in experimental room for 1 hour after their transfer from animal facility. Then, not stressed mice were immediately sacrificed for brain c-fos protein detection (control mice, Cnt). Mice submitted to stress alone (S) or stress and social task (SESI, i.e., stress (S) followed by exploration (E) and social interaction (SI)) were sacrificed either 90 min after the beginning of the stress procedure (Short delay, solid lines) or 90 min after the end of the stress or of the social interaction (Long delay, dotted lines) to allow an identical induction time of c-fos protein between S and SESI groups. ESI mice, i.e., not stress mice submitted to exploration and social interaction were sacrificed 90 min after the end of the social interaction (Long delay, dotted lines). IH mouse submitted to the social task was placed in the experimental box for a 30 min novelty exploration period (E) before to introduce a SV mouse in the box during 8 min to study mice social interactions (SI).

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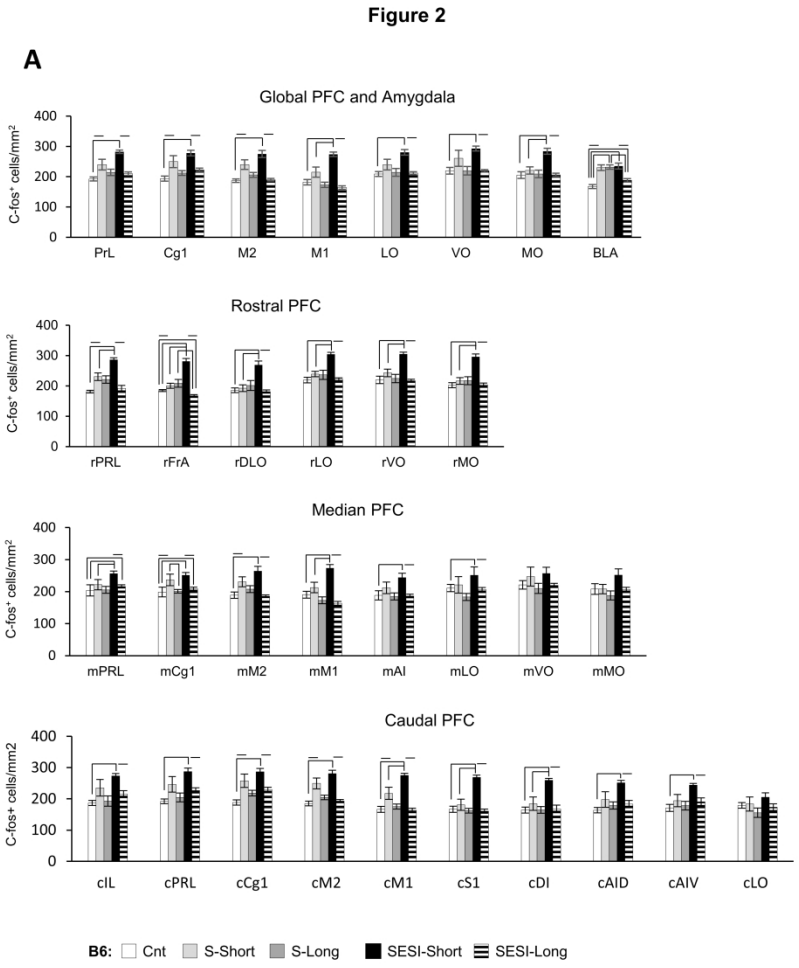

Fig. 2. Stress, social task and time effects on c-fos protein expression. A-B: Quantification of c-fos protein in the PFC and in the BLA in B6 (A) and β2-/- (B) mice subjected to no stress and no behavior (Cnt), to acute stress alone (S mice) and to acute stress followed by social task (SESI mice). Mice were sacrificed either 90 min after the beginning of the stress (S-Short and SESI-Short) or 90 min after the end of the stress or of the social interaction (S-Long and SESI-Long). Histograms are means ± SEM. Significant differences between groups are reported between bars (0.043 ≤ ps < 0.001 for B6 mice, 0.038 ≤ ps < 0.001 for β2-/- mice). PFC and amygdalar sub-areas were distinguished using the atlas of Paxinos and Franklin (2001). Cortical areas were considered either in their extent (from bregma +3.08 to +1.54 mm, Global PFC) or in relation to their rostral (from bregma 3.08 to 2.58 mm, Rostral PFC), median (from bregma 2.46 to 2.10 mm, Median PFC) and caudal (from bregma 1.98 to 1.54 mm, Caudal PFC) location. Letters (r, m, c) preceding the structure's name indicate respectively their rostral, median or caudal level. The BLA was considered as extending from bregma -0.58 to -1.58 mm. AI: agranular insular cortex; AID: agranular insular cortex, dorsal part; AIV: agranular insular cortex, ventral part; BLA: basolateral amygdaloid nucleus, anterior part; Cg1: cingulate cortex, area 1; DI: dysgranular insular cortex; DLO: dorsolateral orbital cortex;

FrA: frontal association cortex; IL: infralimbic cortex; LO: lateral orbital cortex; M1: primary motor cortex; M2: secondary motor cortex; MO: medial orbital cortex; PrL: prelimbic cortex; S1: primary somatosensory cortex; VO: ventral orbital cortex. C-E: Schematic illustrations of c-fos protein immunoreactivity in representative coronal sections of the rostral, median and caudal PFC in B6 and β2-/- mice. Significant increases or decreases of protein expression are indicated by respectively dark and light gray areas (see supplementary Tables 1a and b, 2a and b, 3a and b, for significance of the results). Distance to bregma is pointed on the right of the coronal sections. B6: Cnt, n = 9; S-Short, n = 6; S-Long, n = 7; SESI-Short, n = 8; SESI-Long, n = 10. β2-/-: Cnt, n = 6; S-Short, n = 7; S-Long, n = 7; SESI-Short, n = 8; SESI-Long, n = 7.

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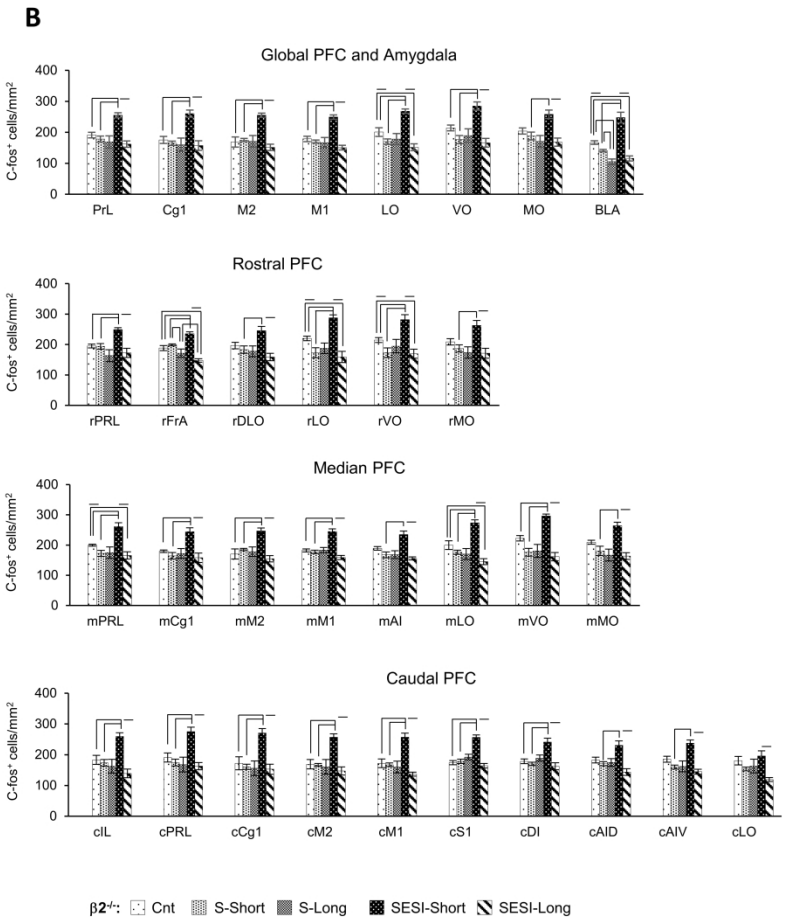
Figure 2



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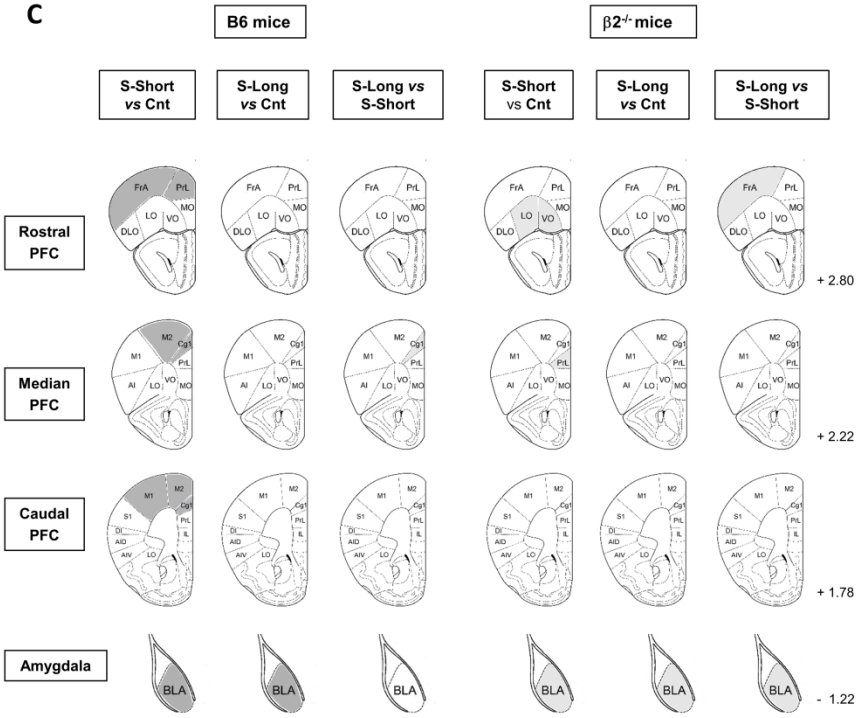

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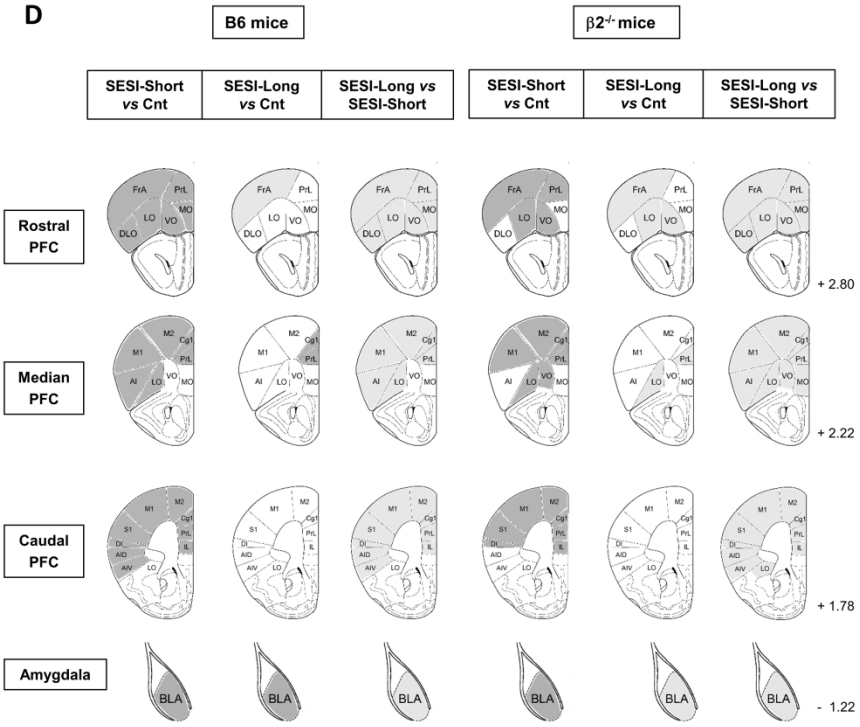

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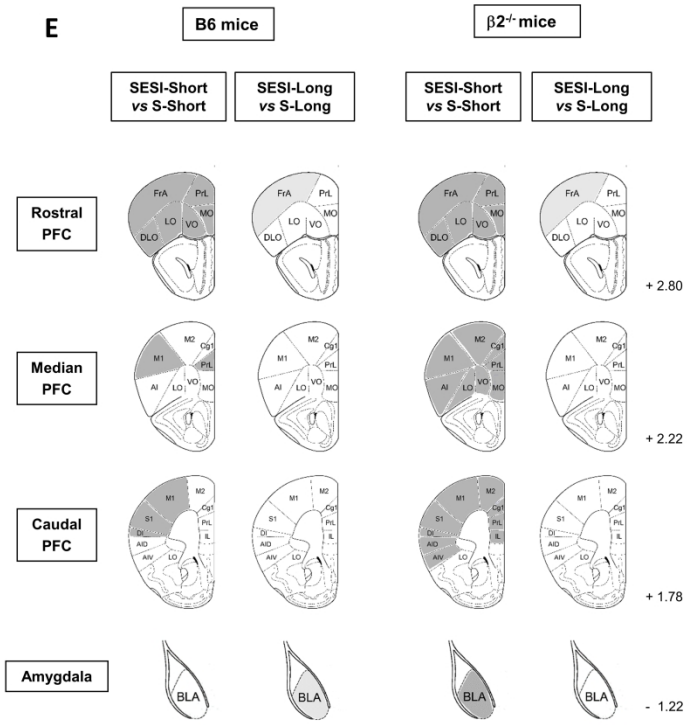

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Figure 3

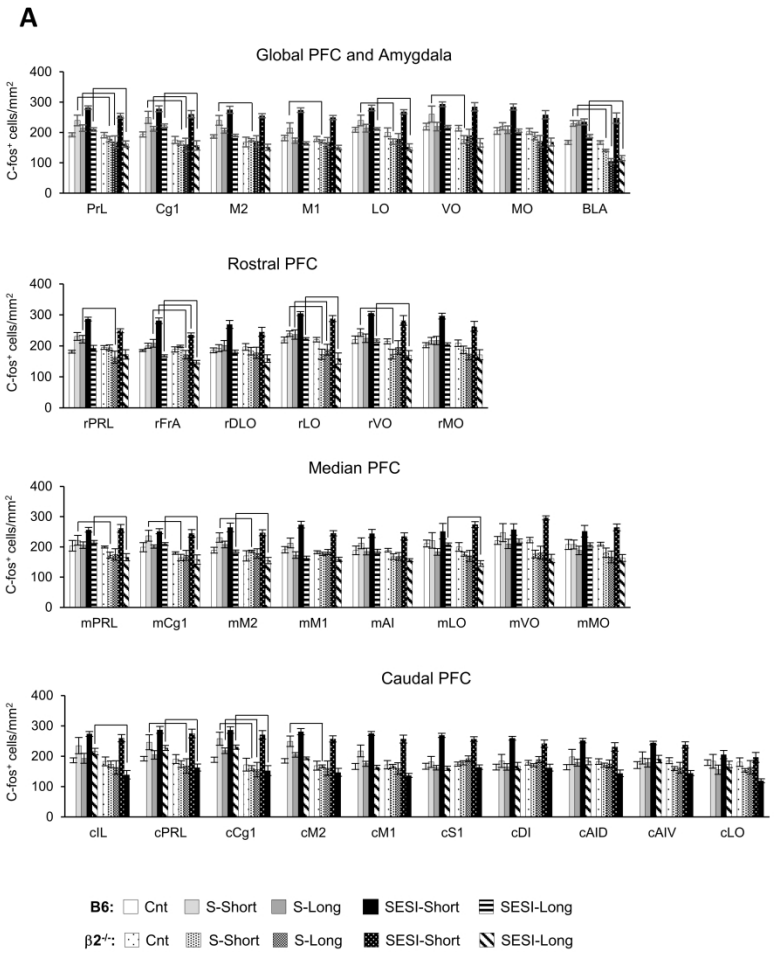

Fig. 3. Genotype effects. β2-/- mice were compared to B6 mice for each experimental procedure, i.e., in control mice (Cnt), after a short and a long delay of c-fos protein expression in mice subjected to stress alone (S-Short and S-Long) and to stress followed by social task (SESI-Short and SESI-Long). Quantification (A) and schematic summary (B) of c-fos protein expression in the PFC and in the BLA. Significant differences are indicated (0.029 ≤ ps ≤ 0.001, see supplementary Tables 4a and b for significance of the results). In the representative coronal sections of the rostral, median and caudal PFC and of the amygdala, light gray areas indicate significant decreases of protein expression. Distance to bregma is reported on the right of the coronal sections (Paxinos and Franklin, 2001). See Fig. 2 for detailed information on brain subdivisions and brain structures abbreviations, and the number of mice used for each group.

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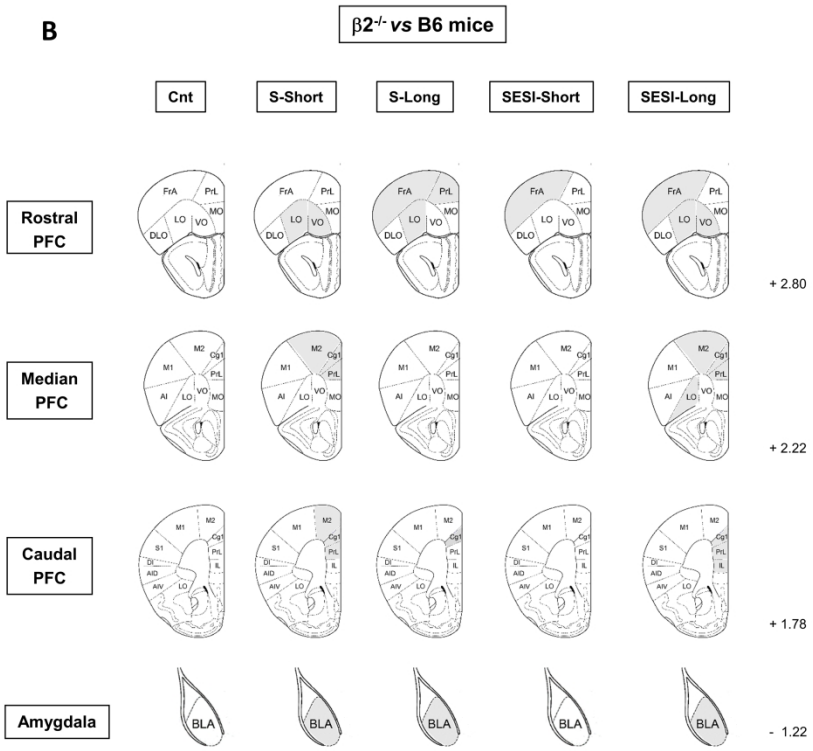
Figure 3



190x253mm (600 x 600 DPI)

Figure 4

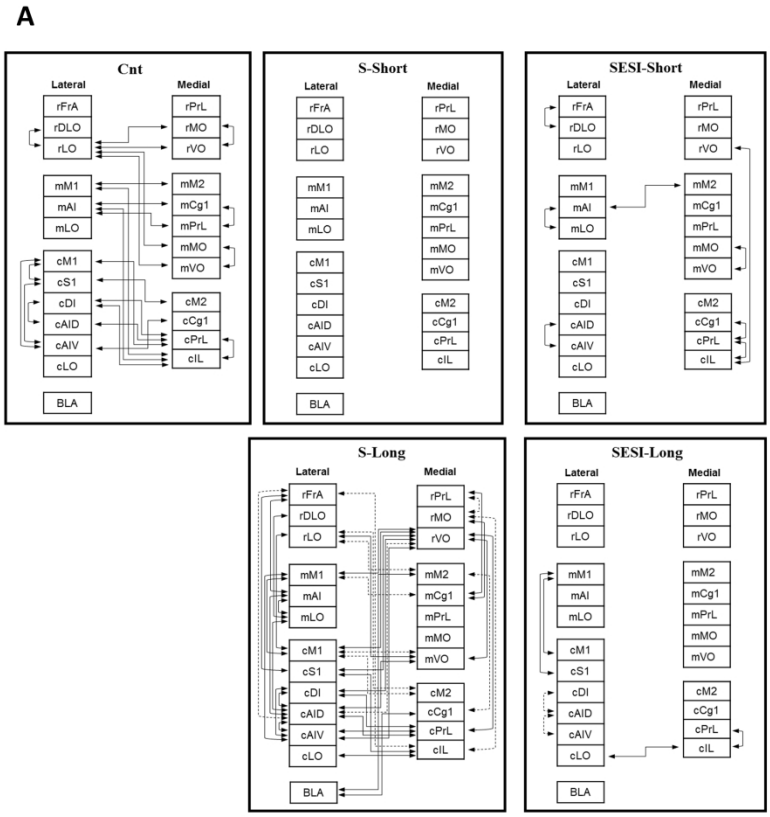
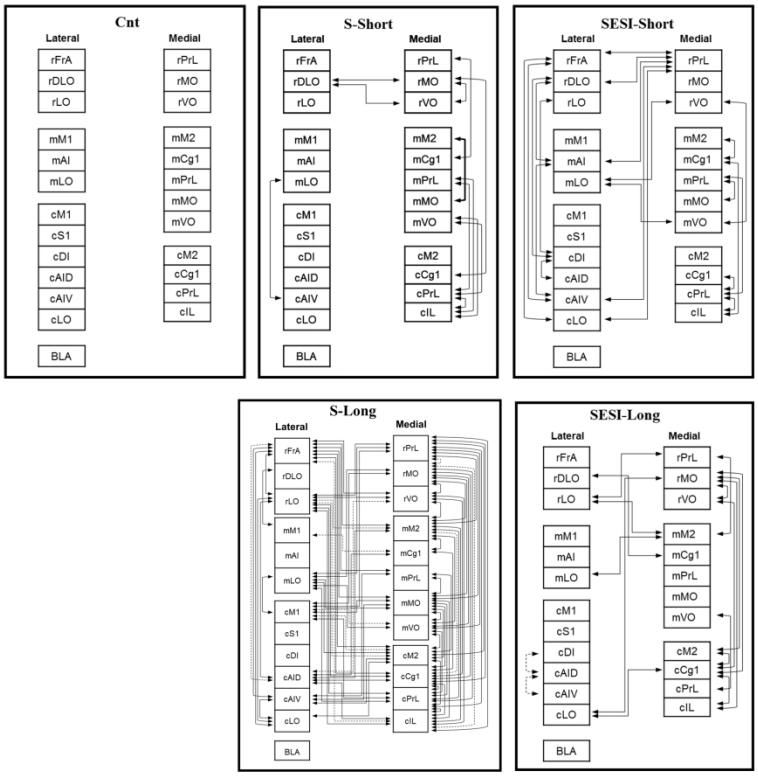


Fig. 4. Correlations between PFC sub-areas and the BLA in B6 (A) and β2-/- mice (B) submitted to different experimental procedures, i.e., in control mice (Cnt), after short and long delay of c-fos protein expression in mice submitted to stress alone (S-Short and S-Long) and to stress and social task (SESI-Short and SESI-Long). Solid arrows indicate positive correlations, bold arrow negative ones. Significant differences following Bonferroni correction are indicated (0.0000002 ≤ ps ≤ 0.00178 for B6 mice, ps = 0.0000002 for β2-/- mice). Dotted arrows indicate correlations common to both genotypes for S-Long and SESI-Long protocols. See Fig. 2 for detailed information on brain subdivisions and brain structures abbreviations, and the number of mice used for each group.

190x253mm (600 x 600 DPI)

Figure 4

B



190x253mm (600 x 600 DPI)

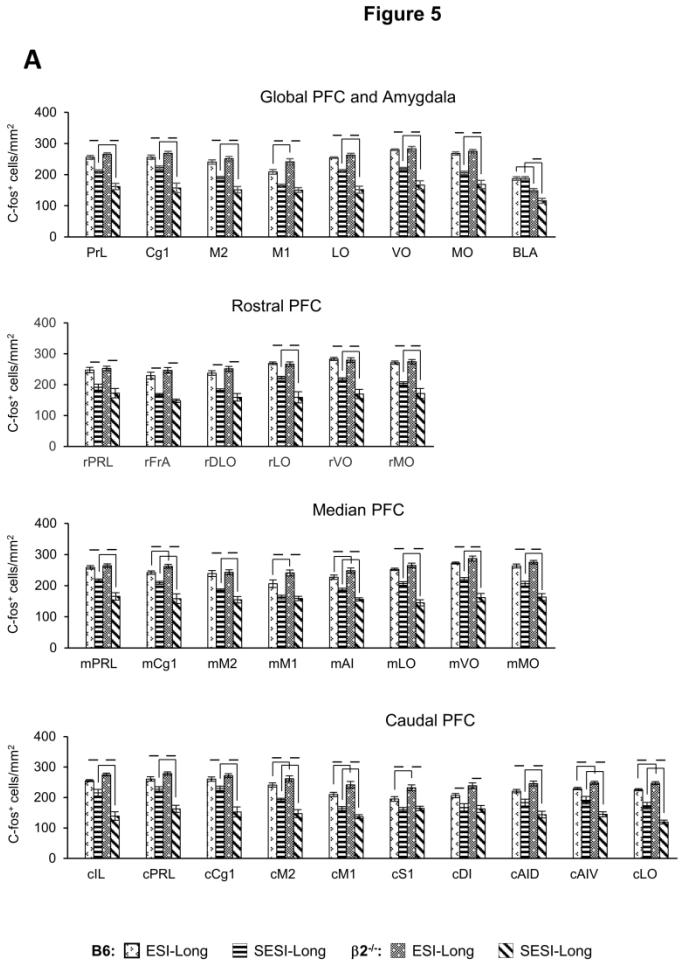
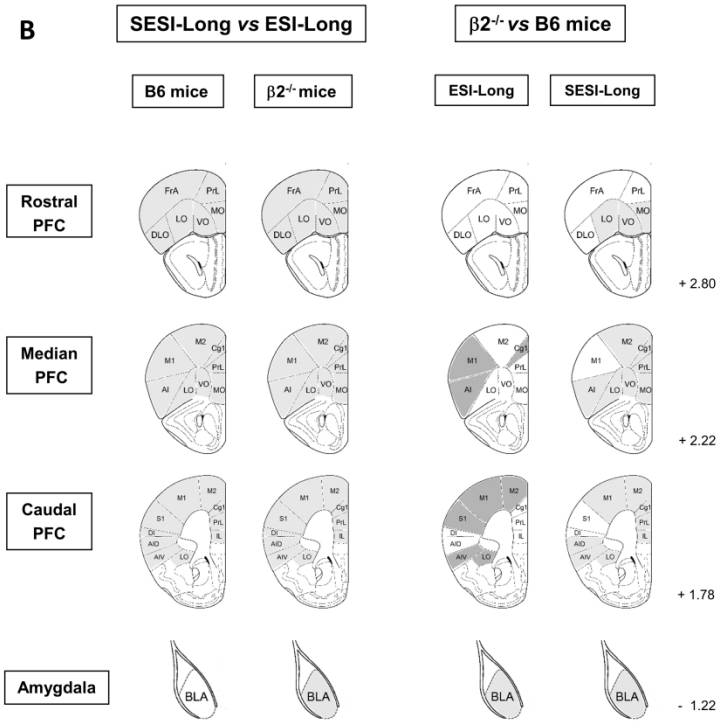


Fig. 5. Stress and social task effects on long term c-fos protein expression in B6 and □2-/- mice. Quantification (A) and schematic illustration (B) of protein expression in the PFC and in the amygdala in stressed and not stressed B6 and □2-/- mice submitted to the social task and sacrificed 90 min after the end of the social interaction (SESI-Long and ESI-Long, respectively). Histograms are means ± SEM. Significant differences are indicated for each genotype between stressed and not stressed mice (0.002 ≤ ps < 0.001 for B6 mice, ps ≤ 0.001 for □2-/- mice), and between genotype for not stressed (0.028 ≤ ps < 0.006 for ESI-Long mice) and stressed mice (0.012 ≤ ps < 0.001 for SESI-Long, see supplementary Table 5a and b for significance of the results). In the representative coronal sections of the rostral, median and caudal PFC and of the BLA, light gray areas indicate significant decreases of protein expression and dark gray areas indicate significant increases. Distances to bregma are indicated on the right of the coronal sections (Paxinos and Franklin, 2001). See Fig. 2 for detailed information on brain subdivisions and brain structures abbreviations. ESI-Long mice: n = 8 for both genotypes, SESI-Long mice: n = 10 B6 and n = 7 □2-/- mice.

190x253mm (600 x 600 DPI)

Figure 5



190x253mm (600 x 600 DPI)

Figure 6.

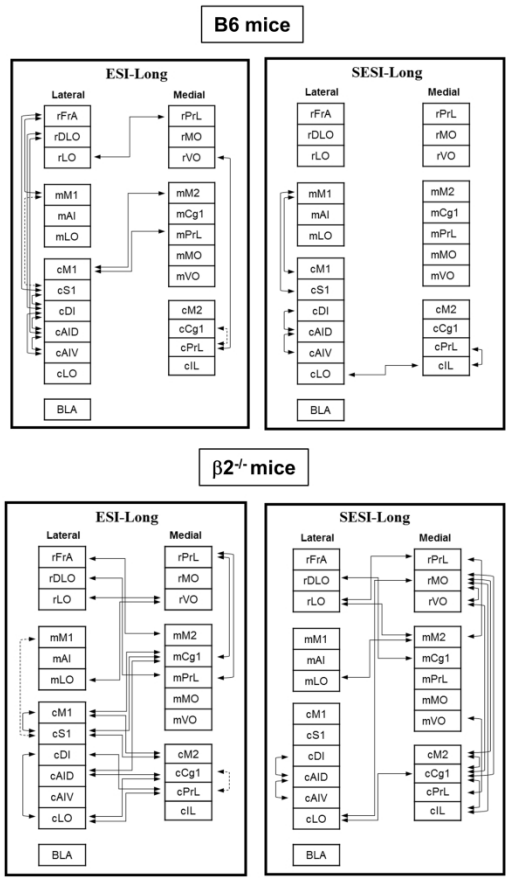


Fig. 6. Correlations between PFC sub-areas and the amygdala in B6 (A) and β2-/- mice (B) submitted or not to acute stress and to the social task. Mice were sacrificed 90 min after the end of social interaction (SESI-Long and ESI-Long, respectively). All correlations (arrows) are positive (statistical thresholds after Bonferroni correction: 0.0000002 ≤ ps ≤ 0.00178 for B6 mice, ps = 0.0000002 for □2-/- mice). Dotted arrows indicate correlations common to both genotypes for S-Long and SESI-Long protocols. See Fig. 2 for detailed information on brain subdivisions and brain structures abbreviations. B6: ESI-Long, n = 8; SESI-Long, n = 10. □2-/: ESI-Long, n = 8; SESI-Long, n = 7.

190x253mm (600 x 600 DPI)

Figure 7

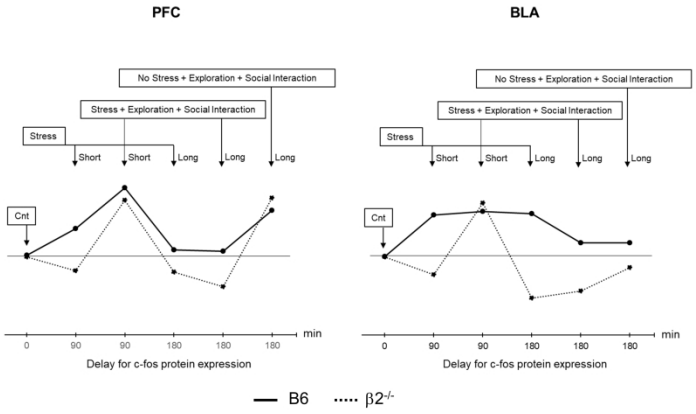


Fig. 7. Schematic representation of the temporal evolution of c-fos protein expression depending on time, behavior and emotional status (stress or not) in the PFC (left) and in the BLA (right) in B6 and □2-/- mice (solid and dotted lines, respectively). C-fos protein was detected 90 (short) and 180 min (Long) after stress alone and after stress coupled with the social task, and 180 min after the social task without stress. Note that in the PFC, both genotypes exhibited similar profiles with a peak of activity when mice were submitted to stress coupled with the social task and sacrificed 90 min after the beginning of stress or when mice, submitted to the social task without stress were sacificed 180 min after the end of the social interaction. By contrast, temporal profile of protein expression in the BLA totally differs between genotypes.

190x253mm (600 x 600 DPI)

Supplementary Table 1a

	Statistical analysis between 10 groups			S-Short *vs* Cnt		S-Long *vs* Cnt		S-Long *vs* S-Short	
				B6	β2$^{-/-}$	B6	β2$^{-/-}$	B6	β2$^{-/-}$
	F or H	*p*	Subsequent test	*p*	*p*	*p*	*p*	*p*	*p*
Global PFC									
PrL	F(9,65) = 16.553	< 0.001	HS	0.030	0.917	0.763	0.796	0.718	0.937
Cg1	F(9,65) = 15.582	< 0.001	HS	0.021	0.951	0.946	0.954	0.407	0.940
M2	F(9,65) = 18.383	< 0.001	HS	0.007	0.988	0.889	0.878	0.339	0.989
M1	F(9,65) = 22.532	< 0.001	HS	0.219	0.996	0.997	0.995	0.059	0.822
LO	H = 52.467, df = 9	< 0.001	MW	0.112	0.035	0.832	0.234	0.138	0.710
VO	F(9,65) = 11.497	< 0.001	HS	0.404	0.625	0.997	0.937	0.482	0.998
MO	F(9,65) = 10.622	< 0.001	HS	0.993	0.992	1.000	0.675	0.996	0.988
Amygdala									
BLA	H = 36.143, df = 9	< 0.001	MW	0.002	0.008	0.001	0.001	1.000	0.007

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Supplementary Table 1b

	Statistical analysis between 10 groups			S-Short *vs* Cnt		S-Long *vs* Cnt		S-Long *vs* S-Short	
				B6	β2$^{-/-}$	B6	β2$^{-/-}$	B6	β2$^{-/-}$
	F or H	*p*	Subsequent test	*p*	*p*	*p*	*p*	*p*	*p*
Rostral PFC									
rPrL	$F_{(9,65)} = 15.010$	< 0.001	HS	0.030	0.949	0.120	0.540	0.959	0.544
rFrA	H = 57.449, df = 9	< 0.001	MW	0.022	0.295	0.072	0.101	1.000	0.026
rDLO	$F_{(9,65)} = 9.010$	< 0.001	HS	1.000	1.000	0.998	0.998	1.000	1.000
rLO	H = 53.706, df = 9	< 0.001	MW	0.316	0.005	0.751	0.138	0.534	0.456
rVO	H = 46.088, df = 9	< 0.001	MW	0.216	0.035	0.832	0.445	0.366	0.318
rMO	$F_{(9,65)} = 11.936$	< 0.001	HS	0.995	0.980	0.997	0.666	0.960	0.994
Median PFC									
mPrL	H = 43.911, df = 9	< 0.001	MW	0.087	0.022	0.397	0.234	0.534	0.902
mCg1	H = 48.526, df = 9	< 0.001	MW	0.029	0.295	0.244	0.534	0.022	1.000
mM2	H = 45.939, df = 9	< 0.001	MW	0.022	0.731	0.169	1.000	0.181	0.620
mM1	$F_{(9,65)} = 19.382$	< 0.001	HS	0.788	0.977	0.901	0.933	0.092	0.994
mAI	$F_{(9,65)} = 7.448$	< 0.001	HS	0.942	0.966	1.000	0.965	0.894	0.997
mLO	H = 40.675, df =9	< 0.001	MW	0.953	0.051	0.138	0.101	0.181	0.805
mVO	$F_{(9,65)} = 8.243$	< 0.001	HS	0.939	0.575	0.997	0.666	0.795	0.999
mMO	$F_{(9,65)} = 6.193$	< 0.001	HS	0.998	0.968	0.993	0.619	0.992	0.999
Caudal PFC									
cIL	$F_{(9,65)} = 10.503$	< 0.001	HS	0.278	0.954	0.927	0.967	0.562	0.986
cPrL	$F_{(9,65)} = 11.498$	< 0.001	HS	0.135	0.986	0.983	0.963	0.559	0.983
cCg1	$F_{(9,65)} = 14.671$	< 0.001	HS	0.012	0.962	0.778	0.978	0.575	0.967
cM2	$F_{(9,65)} = 14.671$	< 0.001	HS	0.003	0.877	0.915	0.977	0.173	0.918
cM1	$F_{(9,65)} = 19.667$	< 0.001	HS	0.029	1.000	1.000	1.000	0.202	1.000
cS1	$F_{(9,65)} = 24.159$	< 0.001	HS	0.994	1.000	1.000	0.976	0.967	0.992
cDI	$F_{(9,65)} = 11.341$	< 0.001	HS	0.993	1.000	0.969	1.000	0.996	0.995
cAID	$F_{(9,65)} = 8.254$	< 0.001	HS	0.657	0.999	0.998	0.999	0.657	0.994
cAIV	$F_{(9,65)} = 8.319$	< 0.001	HS	0.960	0.954	0.998	0.967	0.996	0.890
cLO	$F_{(9,65)} = 3.414$	< 0.001	HS	0.999	0.993	0.994	0.999	0.992	0.999

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Supplementary Table 2a

	Statistical analysis between 10 groups			SESI-Short *vs* Cnt		SESI-Long *vs* Cnt		SESI-Long *vs* SESI-Short	
				B6	β2$^{-/-}$	B6	β2$^{-/-}$	B6	β2$^{-/-}$
	F or H	*p*	Subsequent test	*p*	*p*	*p*	*p*	*p*	*p*
Global PFC									
PrL	$F_{(9,65)} = 16.553$	< 0.001	HS	<0.001	<0.001	0.807	0.573	<0.001	<0.001
Cg1	$F_{(9,65)} = 15.582$	< 0.001	HS	<0.001	<0.001	0.458	0.931	0.008	<0.001
M2	$F_{(9,65)} = 18.383$	< 0.001	HS	<0.001	<0.001	0.951	0.916	<0.001	<0.001
M1	$F_{(9,65)} = 22.532$	< 0.001	HS	<0.001	<0.001	0.876	0.545	<0.001	<0.001
LO	H = 52.467, df = 9	< 0.001	MW	<0.001	<0.001	0.967	0.035	<0.001	<0.001
VO	$F_{(9,65)} = 11.497$	< 0.001	HS	0.002	0.011	1.000	0.312	0.001	<0.001
MO	$F_{(9,65)} = 10.622$	< 0.001	HS	<0.001	0.052	0.997	0.576	<0.001	<0.001
Amygdala									
BLA	H = 36.143, df = 9	< 0.001	MW	<0.001	<0.001	0.030	0.001	<0.001	<0.001

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Supplementary Table 2b

	Statistical analysis between 10 groups			SESI-Short *vs* Cnt		SESI -Long *vs* Cnt		SESI-Long *vs* SESI-Short	
				B6	β2$^{-/-}$	B6	β2$^{-/-}$	B6	β2$^{-/-}$
	F or H	*p*	Subsequent test	*p*	*p*	*p*	*p*	*p*	*p*
Rostral PFC									
rPrL	$F_{(9,65)} = 15.010$	< 0.001	HS	<0.001	0.015	0.967	0.888	<0.001	<0.001
rFrA	H = 57.449, df = 9	< 0.001	MW	<0.001	0.001	0.002	0.002	<0.001	<0.001
rDLO	$F_{(9,65)} = 9.010$	< 0.001	HS	<0.001	0.142	1.000	0.597	<0.001	<0.001
rLO	H = 53.706, df = 9	< 0.001	MW	<0.001	<0.001	0.775	0.005	<0.001	<0.001
rVO	H = 46.088, df = 9	< 0.001	MW	<0.001	0.008	0.307	0.005	<0.001	<0.001
rMO	$F_{(9,65)} = 11.936$	< 0.001	HS	<0.001	0.063	0.988	0.584	<0.001	<0.001
Median PFC									
mPrL	H = 43.911, df = 9	< 0.001	MW	0.008	<0.001	0.037	0.005	0.001	<0.001
mCg1	H = 48.526, df = 9	< 0.001	MW	0.008	0.001	0.045	0.234	<0.001	<0.001
mM2	H = 45.939, df = 9	< 0.001	MW	0.002	0.001	0.838	0.295	<0.001	<0.001
mM1	$F_{(9,65)} = 19.382$	< 0.001	HS	<0.001	<0.001	0.309	0.781	<0.001	<0.001
mAI	$F_{(9,65)} = 7.448$	< 0.001	HS	0.007	0.142	1.000	0.687	0.003	<0.001
mLO	H = 40.675, df =9	< 0.001	MW	0.030	0.001	0.775	0.014	0.011	<0.001
mVO	$F_{(9,65)} = 8.243$	< 0.001	HS	0.659	0.027	0.994	0.173	0.614	<0.001
mMO	$F_{(9,65)} = 6.193$	< 0.001	HS	0.397	0.208	1.000	0.565	0.294	<0.001
Caudal PFC									
cIL	$F_{(9,65)} = 10.503$	< 0.001	HS	<0.001	0.008	0.785	0.506	0.038	<0.001
cPrL	$F_{(9,65)} = 11.498$	< 0.001	HS	<0.001	0.002	0.559	0.921	0.023	<0.001
cCg1	$F_{(9,65)} = 14.671$	< 0.001	HS	<0.001	<0.001	0.232	0.983	0.036	<0.001
cM2	$F_{(9,65)} = 14.671$	< 0.001	HS	<0.001	<0.001	0.982	0.917	<0.001	<0.001
cM1	$F_{(9,65)} = 19.667$	< 0.001	HS	<0.001	<0.001	0.995	0.480	<0.001	<0.001
cS1	$F_{(9,65)} = 24.159$	< 0.001	HS	<0.001	<0.001	1.000	0.998	<0.001	<0.001
cDI	$F_{(9,65)} = 11.341$	< 0.001	HS	<0.001	0.004	1.000	0.999	<0.001	<0.001
cAID	$F_{(9,65)} = 8.254$	< 0.001	HS	<0.001	0.145	0.539	0.539	<0.001	<0.001
cAIV	$F_{(9,65)} = 8.319$	< 0.001	HS	<0.001	0.081	0.961	0.439	0.021	<0.001
cLO	$F_{(9,65)} = 3.414$	< 0.001	HS	0.985	0.999	0.998	0.117	0.874	0.004

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Supplementary Table 3a

	Statistical analysis between 10 groups			SESI-Short *vs* S-Short		SESI-Long *vs* S-Long	
				B6	$\beta2^{-/-}$	B6	$\beta2^{-/-}$
	F or H	*p*	Subsequent test	*p*	*p*	*p*	*p*
Global PFC							
PrL	$F_{(9,65)} = 16.553$	< 0.001	HS	0.085	<0.001	0.934	0.949
Cg1	$F_{(9,65)} = 15.582$	< 0.001	HS	0.700	<0.001	0.978	0.877
M2	$F_{(9,65)} = 18.383$	< 0.001	HS	0.281	<0.001	0.929	0.891
M1	$F_{(9,65)} = 22.532$	< 0.001	HS	<0.001	<0.001	0.997	0.972
LO	H = 52.467, df = 9	< 0.001	MW	0.081	<0.001	0.591	0.456
VO	$F_{(9,65)} = 11.497$	< 0.001	HS	0.755	<0.001	1.000	0.908
MO	$F_{(9,65)} = 10.622$	< 0.001	HS	0.010	0.001	1.000	1.000
Amygdala							
BLA	H = 36.143, df = 9	< 0.001	MW	1.000	<0.001	0.002	0.535

For Peer Review

Supplementary Table 3b

	Statistical analysis between 10 groups			SESI-Short *vs* S-Short		SESI-Long *vs* S-Long	
				B6	β2$^{-/-}$	B6	β2$^{-/-}$
	F or H	*p*	Subsequent test	*p*	*p*	*p*	*p*
Rostral PFC							
rPrL	$F_{(9,65)} = 15.010$	< 0.001	HS	0.010	0.008	0.515	0.979
rFrA	H = 57.449, df = 9	< 0.001	MW	<0.001	<0.001	0.002	0.038
rDLO	$F_{(9,65)} = 9.010$	< 0.001	HS	<0.001	0.008	0.991	0.998
rLO	H = 53.706, df = 9	< 0.001	MW	<0.001	<0.001	0.526	0.456
rVO	H = 46.088, df = 9	< 0.001	MW	<0.001	0.002	0.884	0.456
rMO	$F_{(9,65)} = 11.936$	< 0.001	HS	<0.001	<0.001	0.993	0.998
Median PFC							
mPrL	H = 43.911, df = 9	< 0.001	MW	0.043	<0.001	0.464	0.902
mCg1	H = 48.526, df = 9	< 0.001	MW	0.142	<0.001	0.188	0.710
mM2	H = 45.939, df = 9	< 0.001	MW	0.081	<0.001	0.130	0.318
mM1	$F_{(9,65)} = 19.382$	< 0.001	HS	<0.001	<0.001	0.993	0.694
mAI	$F_{(9,65)} = 7.448$	< 0.001	HS	0.690	0.001	0.999	0.996
mLO	H = 40.675, df =9	< 0.001	MW	0.228	<0.001	0.107	0.318
mVO	$F_{(9,65)} = 8.243$	< 0.001	HS	0.992	<0.001	0.997	0.989
mMO	$F_{(9,65)} = 6.193$	< 0.001	HS	0.551	0.002	0.992	1.000
Caudal PFC							
cIL	$F_{(9,65)} = 10.503$	< 0.001	HS	0.658	<0.001	0.953	0.942
cPrL	$F_{(9,65)} = 11.498$	< 0.001	HS	0.567	<0.001	0.934	0.954
cCg1	$F_{(9,65)} = 14.671$	< 0.001	HS	0.819	<0.001	0.984	0.867
cM2	$F_{(9,65)} = 14.671$	< 0.001	HS	0.611	<0.001	0.972	0.970
cM1	$F_{(9,65)} = 19.667$	< 0.001	HS	0.012	<0.001	0.999	0.920
cS1	$F_{(9,65)} = 24.159$	< 0.001	HS	<0.001	<0.001	0.875	0.477
cDI	$F_{(9,65)} = 11.341$	< 0.001	HS	<0.001	<0.001	1.000	0.931
cAID	$F_{(9,65)} = 8.254$	< 0.001	HS	0.062	0.014	0.999	0.803
cAIV	$F_{(9,65)} = 8.319$	< 0.001	HS	0.122	<0.001	0.995	0.995
cLO	$F_{(9,65)} = 3.414$	< 0.001	HS	0.998	0.599	0.999	0.604

For Peer Review

Supplementary Table 4a

	Statistical analysis between 10 groups			$\beta2^{-/-}$ *vs* B6				
				Cnt	S-Short	S-Long	SESI-Short	SESI-Long
	F or H	*p*	Subsequent test	*p*	*p*	*p*	*p*	*p*
Global PFC								
PrL	$F_{(9,65)} = 16.553$	< 0.001	HS	0.916	0.003	0.049	0.527	0.013
Cg1	$F_{(9,65)} = 15.582$	< 0.001	HS	0.937	<0.001	0.048	0.937	0.001
M2	$F_{(9,65)} = 18.383$	< 0.001	HS	0.915	<0.001	0.291	0.893	0.093
M1	$F_{(9,65)} = 22.532$	< 0.001	HS	0.992	0.029	0.997	0.602	0.976
LO	H = 52.467, df = 9	< 0.001	MW	0.953	0.001	0.097	0.382	<0.001
VO	$F_{(9,65)} = 11.497$	< 0.001	HS	1.000	0.002	0.779	0.999	0.075
MO	$F_{(9,65)} = 10.622$	< 0.001	HS	0.949	0.702	0.430	0.838	0.348
Amygdala								
BLA	H = 36.143, df = 9	< 0.001	MW	0.680	0.001	<0.001	0.574	<0.001

For Peer Review

Supplementary Table 4b

	Statistical analysis between 10 groups			β2$^{-/-}$ *vs* B6				
				Cnt	S-Short	S-Long	SESI-Short	SESI-Long
	F or H	*p*	Subsequent test	*p*	*p*	*p*	*p*	*p*
Rostral PFC								
rPrL	$F_{(9,65)} = 15.010$	< 0.001	HS	0.976	0.364	0.006	0.179	0.860
rFrA	H = 57.449, df = 9	< 0.001	MW	0.517	0.731	0.038	<0.001	0.017
rDLO	$F_{(9,65)} = 9.010$	< 0.001	HS	1.000	1.000	0.978	0.969	0.977
rLO	H = 53.706, df = 9	< 0.001	MW	0.768	0.001	0.038	0.105	0.003
rVO	H = 46.088, df = 9	< 0.001	MW	0.517	0.008	0.318	0.195	<0.001
rMO	$F_{(9,65)} = 11.936$	< 0.001	HS	0.997	0.819	0.272	0.584	0.587
Median PFC								
mPrL	H = 43.911, df = 9	< 0.001	MW	0.377	0.008	0.165	1.000	0.002
mCg1	H = 48.526, df = 9	< 0.001	MW	0.377	0.002	0.097	0.878	0.002
mM2	H = 45.939, df = 9	< 0.001	MW	0.596	0.001	0.165	0.382	0.028
mM1	$F_{(9,65)} = 19.382$	< 0.001	HS	0.992	0.211	0.996	0.360	0.990
mAI	$F_{(9,65)} = 7.448$	< 0.001	HS	0.973	0.204	0.980	0.997	0.646
mLO	H = 40.675, df =9	< 0.001	MW	0.517	0.138	0.535	0.645	0.001
mVO	$F_{(9,65)} = 8.243$	< 0.001	HS	0.954	0.051	0.911	0.643	0.102
mMO	$F_{(9,65)} = 6.193$	< 0.001	HS	1.000	0.973	0.995	0.998	0.456
Caudal PFC								
cIL	$F_{(9,65)} = 10.503$	< 0.001	HS	0.854	0.092	0.861	0.983	0.002
cPrL	$F_{(9,65)} = 11.498$	< 0.001	HS	0.941	0.018	0.678	0.971	0.016
cCg1	$F_{(9,65)} = 14.671$	< 0.001	HS	0.981	<0.001	0.041	0.987	0.001
cM2	$F_{(9,65)} = 14.671$	< 0.001	HS	0.952	<0.001	0.164	0.820	0.058
cM1	$F_{(9,65)} = 19.667$	< 0.001	HS	1.000	0.059	0.997	0.977	0.688
cS1	$F_{(9,65)} = 24.159$	< 0.001	HS	0.999	0.995	0.358	0.993	0.999
cDI	$F_{(9,65)} = 11.341$	< 0.001	HS	1.000	1.000	0.966	0.996	1.000
cAID	$F_{(9,65)} = 8.254$	< 0.001	HS	0.993	0.941	0.999	0.978	0.276
cAIV	$F_{(9,65)} = 8.319$	< 0.001	HS	0.995	0.696	0.996	0.990	0.101
cLO	$F_{(9,65)} = 3.414$	< 0.001	HS	0.926	0.985	0.999	1.000	0.124

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Supplementary Table 5a

	Statistical analysis between 4 groups			SESI-Long *vs* ESI-Long		β2$^{-/-}$ *vs* B6	
				B6	β2$^{-/-}$	ESI-Long	SESI-Long
	F or H	*p*	Subsequent test	*p*	*p*	*p*	*p*
Global PFC							
PrL	H = 26.864, df = 3	< 0.001	MW	< 0.001	< 0.001	0.195	0.002
Cg1	H = 26.051, df = 3	< 0.001	MW	0.005	<0.001	0.083	0.001
M2	$F_{(3,32)}$ = 48.002	< 0.001	HS	<0.001	<0.001	0.284	<0.001
M1	$F_{(3,32)}$ = 34.044	< 0.001	HS	<0.001	<0.001	0.006	0.182
LO	H = 27.706, df = 3	< 0.001	MW	< 0.001	0.001	0.161	<0.001
VO	H = 27.225, df = 3	< 0.001	MW	< 0.001	< 0.001	0.721	<0.001
MO	$F_{(3,32)}$ = 49.270	< 0.001	HS	<0.001	<0.001	0.510	0.001
Amygdala							
BLA	$F_{(3,32)}$ = 31.413	< 0.001	HS	0.952	0.002	<0.001	<0.001

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Supplementary Table 5b

	Statistical analysis between 4 groups			SESI-Long *vs* ESI-Long		$\beta2^{-/-}$ *vs* B6	
				B6	$\beta2^{-/-}$	ESI-Long	SESI-Long
	F or H	*p*	Subsequent test	*p*	*p*	*p*	*p*
Rostral PFC							
rPrL	$F_{(3,32)} = 16.412$	< 0.001	HS	<0.001	<0.001	0.703	0.297
rFrA	$F_{(3,32)} = 43.838$	< 0.001	HS	<0.001	<0.001	0.094	0.102
rDLO	$F_{(3,32)} = 34.723$	< 0.001	HS	<0.001	<0.001	0.204	0.075
rLO	H = 25.706, df = 3	< 0.001	MW	<0.001	<0.001	0.798	0.003
rVO	H = 26.960, df = 3	< 0.001	MW	<0.001	<0.001	0.721	<0.001
rMO	$F_{(3,32)} = 36.986$	< 0.001	HS	<0.001	<0.001	0.809	0.015
Median PFC							
mPrL	H = 26.487, df = 3	< 0.001	MW	<0.001	<0.001	0.442	0.002
mCg1	H = 26.937, df = 3	< 0.001	MW	0.002	<0.001	0.028	0.002
mM2	$F_{(3,32)} = 31.219$	< 0.001	HS	<0.001	<0.001	0.718	0.013
mM1	H = 24.451, df = 3	< 0.001	MW	0.001	<0.001	0.010	0.807
mAI	$F_{(3,32)} = 39.367$	< 0.001	HS	<0.001	<0.001	0.028	0.004
mLO	$F_{(3,32)} = 66.234$	< 0.001	HS	<0.001	<0.001	0.176	<0.001
mVO	H = 26.864, df = 3	< 0.001	MW	<0.001	<0.001	0.161	0.002
mMO	$F_{(3,32)} = 47.799$	< 0.001	HS	<0.001	<0.001	0.257	<0.001
Caudal PFC							
cIL	$F_{(3,32)} = 43.271$	< 0.001	HS	0.004	<0.001	0.114	<0.001
cPrL	$F_{(3,32)} = 38.701$	< 0.001	HS	0.007	<0.001	0.115	<0.001
cCg1	H = 24.215, df = 3	< 0.001	MW	0.004	<0.001	0.195	0.002
cM2	H = 26.504, df = 3	< 0.001	MW	<0.001	<0.001	0.050	0.004
cM1	$F_{(3,32)} = 37.173$	< 0.001	HS	<0.001	<0.001	0.012	0.018
cS1	$F_{(3,32)} = 20.380$	< 0.001	HS	0.005	<0.001	0.007	0.722
cDI	$F_{(3,32)} = 12.906$	< 0.001	HS	0.022	<0.001	0.059	0.716
cAID	$F_{(3,32)} = 22.630$	< 0.001	HS	0.009	<0.001	0.063	0.012
cAIV	H = 24.764, df = 3	< 0.001	MW	0.005	<0.001	0.015	0.013
cLO	H = 27.992, df = 3	< 0.001	MW	<0.001	<0.001	0.015	0.005

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Supplementary Captions to Tables.

Supplementary Table 1. Statistical analyses in B6 and β2$^{-/-}$ mice submitted to acute stress (**S**) when c-fos protein was detected 90 min after the beginning (short delay, **S-Short**) or the end of the stress procedure (long delay, **S-Long**; see Fig. 1). For each genotype, comparisons were done with their respective controls (**Cnt**) and between S-Long and S-Short protocols. Comparisons were performed on 10 independent groups with Kruskal-Wallis one-way ANOVA on Ranks *(p)* in the Global PFC and in the amygdala **(a)**, and in the rostral, median and caudal PFC **(b).** When normality passed, data were subsequently analyzed with the Holm-Sidak method test (F) and when data did not follow a Gaussian distribution we performed Mann-Whitney U-tests (H). *p* of post-hoc comparisons are indicated (post-hoc). Gray areas indicated significant differences.

Supplementary Table 2. Statistical analyses in B6 and β2$^{-/-}$ mice submitted to stress followed by exploration and social interaction (**SESI**) when c-fos protein was detected 90 min after the beginning of stress procedure (short delay, **SESI-Short**) or 90 min after the end of social interaction (long delay, **SESI-Long**; see Fig. 1). For each genotype, comparisons were done with their respective controls (**Cnt**) and between SESI-Long and SESI-Short protocols. Comparisons were performed on 10 independent groups using Kruskal-Wallis one way ANOVA on Ranks *(p)* in the Global PFC and in the amygdala **(a)** and in the rostral, median and caudal PFC **(b).** See supplementary Table 1 for statistical comparisons. Gray areas indicated significant differences.

Supplementary Table 3. Statistical analyses in B6 and β2$^{-/-}$ mice submitted to stress alone (**S**) and to stress followed by exploration and social interaction (**SESI**). For each genotype, comparisons were done when c-fos protein was detected 90 min after the beginning of stress (short delay, **SESI-Short** vs **S-Short**) and 90 min after the end of stress or social interaction (long delay, **SESI-Long** vs **S-Long**; see Fig. 1). Statistical analyses were performed on 10 independent groups using Kruskal-Wallis one-way ANOVA on Ranks *(p)* in the Global PFC and in the amygdala **(a)** and in the rostral, median and caudal PFC **(b).** See supplementary Table 1 for statistical comparisons. Gray areas indicated significant differences.

Supplementary Table 4. Statistical comparisons between B6 and β2$^{-/-}$ mice submitted to no stress and no behavior (Control mice, **Cnt**), to stress alone (**S**) with c-fos protein detected 90 min after the beginning of stress (short delay, **S-Short**), 90 min after the end of stress (long delay, **S-Long**), 90min after the beginning

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of stress followed by exploration and social interaction (**SESI short**), and 90min after the end of social interaction (**SESI-Long**) (see Fig. 1). Statistical analyses were performed on 10 independent groups using Kruskal-Wallis one way ANOVA on Ranks *(p)* in the Global PFC and in the amygdala **(a)** and in the rostral, median and caudal PFC **(b).** See supplementary Table 1 for statistical comparisons. Gray areas indicated significant differences.

Supplementary Table 5. Statistical analyses in B6 and $\beta2^{-/-}$ mice submitted to exploration and social interaction following or not acute stress (**SESI** and **ESI** mice, respectively) when c-fos protein was detected 90 min after the end of the social interaction (long delay, see Fig. 1B). Comparisons were done between **SESI-Long** and **ESI-Long** mice for each genotype (middle columns) and between B6 and $\beta2^{-/-}$ for each experimental condition ($\beta2^{-/-}$ versus B6 mice, ESI-Long and SESI-Long, right columns). Comparisons were performed on 4 independent groups with Kruskal-Wallis one-way ANOVA on Ranks *(p)* in the Global PFC and in the amygdala **(a)**, and in the rostral, median and caudal PFC **(b).** See supplementary Table 1 for statistical comparisons. Gray areas indicated significant differences.

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