Social Transmission of Fear

Thesis

Social transmission of fear: The effect of social interaction pattern on fear conditioning by-proxy and the role of β2-subunit of nicotinic acetylcholine receptors

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Preface

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Abstract

Applying models of observational fear learning to genetically modified rodents would facilitate the study of the neural mechanisms underlying the social transmission of fear-related information. Here, we examined fear conditioning by-proxy (FCbP) in normal animals and animals lacking the β2 subunit of the nicotinic Acetylcholine receptor (nAChR). Naïve C57BL_6 and β2−/− mice (FCbP) were exposed to a previously fear-conditioned (FC) cage-mate during the presentation of the conditioned stimulus (tone; Day 2). On the following day FCbP mice were tested for fear reactions to both tone and context (Day 3) and we assessed the contribution of several factors to the estimated fear response. Although FCbP animals of both genotypes displayed no contextual fear, they showed significant differences in cued-fear: β2−/− mice did not freeze to the stimulus, while 30% wild-type mice expressed cued fear. Interestingly, only wt mice that exhibited enhanced social interaction with the FC animal during tone presentation (Day 2) expressed fear to the tone (Day 3). These results suggest that (i) mice are able to acquire information about possible danger through social interaction; (ii) the efficiency of social transmission of fear depends on the interaction pattern between animals during cue presentation; and (iii) β2−/− mice display different interaction pattern compared to wt mice and are unable to acquire such information. These data indicate that β2-containing AChRs influence observational fear learning indirectly, through their effect on social behavior.
Introduction

Reacting promptly in the presence of threats is critical to survival. The ability to identify cues that predict danger greatly supports us in this direction. For example, warning signs promote road safety by alerting road users to conditions that might call for a certain action (reduction of speed, cautious driving, etc). In other words, traffic signs forewarn the drivers about potentially harmful events and help them generate appropriate behaviors in order to avoid the hazard.

Learning about potentially harmful events permits us to establish associations between external cues and emotional/motivational states such as fear. Fear can be characterized by anxiety and agitation due to the expectation of impending danger and thus serves as an adaptive alert mechanism for the organism. It can be acquired through direct experiences or indirectly, through social transmission (Olsson et al., 2007). For example, you might fear a certain sharp turn because you had a car accident there, because you saw someone else having an accident there or because somebody told you about a severe crash in that turn. In all cases, your fear might express itself similarly, such as by avoidance of this particular turn or by very cautious driving and increased autonomic arousal (such as sweating and increased heart rate) when approaching it.

Much of our knowledge regarding the neurobiological mechanisms of fear learning comes from an extensive animal literature on Pavlovian (classical) fear conditioning - an established model of direct fear learning. A typical fear conditioning paradigm involves the association of a neutral stimulus (i.e., a tone; conditioned stimulus, CS) which has no effect on animals with a naturally aversive stimulus (i.e., a foot shock; unconditioned stimulus, US) which elicits fear responses, such as autonomic (i.e., changes in heart rate) or behavioral (i.e., immobility, jump). After repeated temporal associations of the tone with shock (i.e., delivery
of shock during the last seconds of the presentation of tone), presentation of the tone by itself elicits a conditioned fear response (i.e., immobility). Similarly, repeated associations of a particular context (e.g. a chamber with a given odor and illumination) with shock can lead to fear responses to that context. In this case, fear responses are elicited by a combination of environmental stimuli rather by a particular cue. It is now believed that Pavlovian fear conditioning evokes the generation of conditioned fear responses to the CS, due to the information that the CS provides about the occurrence of the US (Rescorla, 1988). To put it differently, animals learn to fear stimuli that predict danger - acquiring information about the CS-US relation. The consistency in the physiological expression of conditioned fear elicited by the basic protocol indicates that mechanisms of emotional learning are analogous across species (LeDoux, 1996). More importantly, neuropsychological and neuroimaging techniques in the research of human fear conditioning have replicated the existing animal models (Delgado et al., 2006).

Although animal models of fear conditioning have been proven valuable in describing human fear conditioning, humans extensively use a less risky way to learn about threats, namely social transmission of information about danger (i.e., through language or observation of fear signals in others). In fact, social transmission and detection of fear signals have been documented in various species, such as birds, mice, rats, cats, non-human primates and humans (Delgado et al., 2006). It has been suggested that fear signs alert the observer about potential danger and “assign a threat value to the context or cue associated with the threat” (Olsson and Phelps, 2007, p.1096). In fear conditioning terms, a conspecific’s fear expression might serve as an aversive stimulus (US) which evokes fear responses to the observer and becomes associated with a paired neutral stimulus (CS). Actually, several indications suggest that observational fear learning relies considerably on the same basic associative learning processes as classical fear conditioning - although it seems to show greater interspecies
variability (e.g. in contrast to humans, social learning in rats do not replicate some core features of classical fear conditioning such as blocking, overshadowing and latent inhibition; Olsson and Phelps, 2007).

Different lines of research inform the study of fear learning through social observation. In this study, we focus on rodent literature since rodents have been used extensively in the research of fear conditioning. Rodent literature on observational fear learning indicates that these animals are sensitive to the distress of others, and that experience with a distressed conspecific can modulate how a rodent subsequently learns about environmental cues and contexts that predict fearful situations (Panksepp and Lahvis, 2011). As discussed below, there is now evidence suggesting that a rodent can express fear to a CS (cue or context) that has been associated with the distress of a conspecific.

In this line of research, employing a contextual conditioning paradigm, Jeon et al. (2010) demonstrated that observer mice express freezing behavior in the context (CS) where 24h earlier they observed a demonstrator mouse receiving repeated foot-shocks. Freezing is defined as the absence of all visible movement except for breathing. It is a robust response in rodents and a highly sensitive measure of fear. In other words, the freezing behavior of the observer in the given context reflects observational fear learning. Interestingly, Jeon et al. (2010) demonstrated that the magnitude of fear response is dependent on relatedness or familiarity of the observer to the demonstrator suggesting that social relationship is an important element in observational fear conditioning in mice.

In an earlier study, observer mice experienced the distress of conspecifics undergoing a series of tone-shock pairings (cued conditioning paradigm; Chen et al., 2009). During playback of the tone (CS) observer mice expressed freezing behavior. Similarly to Jeon et al. (2010), this study showed that observer mice were responsive to environmental cues that predict distress in others. Interestingly, the researchers reproduced this freezing behavior to
the CS presentation by exposing the observer mice to pairings of the CS and playbacks of conspecific distress vocalizations in the absence of demonstrators. This finding suggests that vocal communication is critical to the social transfer of fear among mice. In addition, Chen et al. (2009) showed that the genetic background could influence the degree to which a mouse is responsive to the distress in others, since fear response was recorded only in one of the two genotypes tested, namely the C57BL/6J strain (B6), but not the BALB/cJ one (BALB). Furthermore, only B6 observer mice exhibited physiological correlates of empathy (namely, heart rate deceleration) while they were experiencing conspecific distress. The B6 and BALB mouse strains have been used by several laboratories as an experimental model of sociability with B6 mice representing a sociable strain and BALB a possible mouse model of autism. Thus, B6 mice might be an appropriate strain to explore the mechanisms underlying the positive association between observational fear learning and sociability.

The studies mentioned above indicate that mice learned to fear the CS experiencing (visual, olfactory, auditory stimuli) conspecifics undergo several CS-US pairings (contextual or cued fear conditioning). On the other hand, two recent studies in rats examined the effect of the conditioned response of a fear-conditioned demonstrator on the observer’s freezing response to the CS (Kim et al., 2010; Bruchey et al., 2010). Therefore, these studies explored observational fear learning in the absence of the threat (US), based only on the demonstrators’ fearful response to the CS (predictor of the threat). In more detail, the demonstrator underwent fear conditioning to a tone and the next day it was presented with the tone in the presence of an observer (Kim et al., 2010; Bruchey et al., 2010).

Kim et al. (2010) showed that observer rats acquired a freezing response to the tone only if they had prior fear experiences themselves (experience with unsignaled foot-shocks). Thus, in this paradigm, a CS triggered a conditioned fear response in demonstrators, which in turn engendered a fear response, but only in “experienced” observers. On the other hand,
Bruchey et al. (2010) demonstrated that some observer rats with no direct fear experience expressed appreciable freezing responses when they were presented with the tone. Interestingly, this latter study demonstrated a positive correlation between the magnitude of the observers’ freezing response to the CS and the amount of social interaction they displayed towards demonstrators during the observation session. Thus, in this experiment, a CS that predicted distress in a familiar demonstrator engendered a fear response only in observers that displayed high levels of social interaction with the demonstrator.

Overall, the experiments considered above demonstrate that even rodents do not require direct experience of a CS-US relation in order to express fear to a cue/context that predicts danger. Rather, naïve observers responded to environmental cues that predict distress in others. These studies implicate several variables that appear to modulate rodent sensitivity to other’s distress, including genotype (Chen et al., 2009), familiarity (Jeon et al., 2010) and the amount of social interaction (Bruchey et al., 2010). In the present study, we wanted to replicate - and extend - Bruchey’s (2010) findings using mice. Particularly, our aim was to explore in depth the role of social interaction in fear conditioning by-proxy (a paradigm of observational fear learning; mainly based on Bruchey et al., 2010), as well as the underlying neurobiological mechanisms. For this purpose, we decided to use genetically modified mice that display impairments in social interaction and/or damage in brain areas correlated with social interaction. Mice lacking the β2 subunit of nicotinic acetylcholine receptors (β2−/− nAChRs) seemed to match our criteria.

nAChRs are ligand-gated ion channels are widely distributed in the brain and are implicated in modulating central nervous system functions (for a review; Dos-Santos Coura and Granon, 2012). Brain nAChRs are pentameric oligomers composed of protein subunits (12 subunits: α2–α10 and β2–β4) arranged in various combinations of α and β. The molecular characterization of these proteins and the emergence of transgenic animal models have
highlighted the role of nAChRs in cognitive functions (Dos-Santos Coura and Granon, 2012). Particularly, studies in knockout mice (β2−/−; mice lacking the gene encoding β2 subunit) demonstrated an important role of the β2-containing receptors in cognitive and executive processes (Granon et al. 2003; Avale et al. 2011; Bourgeois et al. 2011; Guillem et al., 2011; Granon and Changeux, 2012).

Mice lacking the β2-containing receptors display (1) morphological alterations in neurons of the cingulate cortex (CC; Konsolaki and Skaliata, submitted; deFelipe paper) - a brain area correlated with social interaction (in humans, non-human primates and rodents; for a review; Rudebeck et al., 2008; Hadland et al., 2003) and observational fear learning (in humans and mice; Olsson et al., 2007; Scearce-Levie et al., 2008), (2) increased social interaction after social isolation, which is restored to normal levels after re-expression of the β2-subunit in the prelimbic (PrL) area of the prefrontal cortex (PFC; Avale et al., 2011), and (3) impaired behavioural flexibility, which resembles the effects of brain damage in the CC and PFC (Granon et al., 2003; Ragozzino and Rozman, 2007; Serreau et al., 2011; Avale et al., 2011). Overall, β2 knockout mice (ko) show impairments in the brain areas that have been correlated with social interaction (namely, CC and PrL of PFC), and impaired social interaction and behavioural flexibility. Consequently, we decided to explore the role of social interaction in observational fear learning using these transgenic mice.

We aimed to test three hypotheses using the fear conditioning by-proxy (FCbP) paradigm. Particularly, we tested whether (1) mice can acquire fear about a CS based only on a conspecific’s fear response to that stimulus, (2) social transfer of fear depends on the social interaction the observer mice display towards the demonstrator, and (3) social transfer of fear is affected by the lack of the β2-subunit of nAChRs. We used B6 (wild type and β2 ko) mice and formed each “observer-demonstrator pair” using animals of the same cage, in order to control the genotype and the familiarity effect, respectively.
Materials and Methods

Animals and housing

The study was performed in the animal facility of the Center for Experimental Surgery of the Biomedical Research Foundation of the Academy of Athens, and was evaluated and authorized by the Veterinary Service of the Prefecture of Athens, as required by the Greek legal requirements for animal experimentation.

A total number of 63 male C57BL/6J mice (B6) at the age of 3.5 to 4.5 months old were studied (body weight: 25-35gr). In more detail, the sample consisted of 27 β2 knockout (β2 ko) and 36 wild type (wt) mice. All animals were obtained from the breeding colony of the animal facility of the Foundation and were housed at a room temperature of 24 ± 2 °C, a relative humidity of 55 ± 10% and a 12h:12h light/dark cycle (07:00/19:00). Animals were maintained according to the Guide for the Care and Use of Laboratory Animals and the relevant recommendations of the European Commission on the care and use of laboratory animals.

Fifteen days prior to the experiment the animals were randomly divided into triads and housed in H-Temp™ polysulfone type III cages (365 mm (L) × 207 mm (W) × 185 mm (H), H-Temp™, Tecniplast, Buguggiate, Varese, Italy). The bedding in each cage comprised of corncob bedding (Rehofix MK 2000, J. Rettenmaier & So, Rosenberg, Germany). The cages were cleaned once a week. All animals had ad libitum access to filtered tap water in drinking bottles and a pelleted chow that contained 18,5 % protein, 5,5 % fat, 4,5 % fiber, 6 % ash (irradiated vacuum packed, 2918, Harlan, Italy).

Experimental Design

Animals were housed in triads for 2-4 weeks prior to the beginning of the experiment. Each triad comprised of (a) a demonstrator that would undergo classical fear conditioning
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(fear conditioned animal, FC), (b) an observer that would undergo fear conditioning by-proxy (fear conditioned by-proxy, FCbP), και (c) a naïve control (not fear-conditioned, naïve). The animals of each cage were randomly marked as FC, FCbP and N the first day of the experiment. All mice were habituated to the behavioural testing room, the transport and the experimenter’s hand for 5 days prior to the beginning of the experiment. All the behavioural tests were conducted during the light phase of the animal light/dark cycle (0700–1900 h), at a room temperature of 22±1°C and a low-level illumination (30 lux).

In order to test our hypotheses we conducted a 3-day experimental protocol (FCbP paradigm). The first day, the demonstrator of each triad (FC) underwent fear conditioning to a tone (5 tone-shock pairings). The next day, the FC mouse was returned to the conditioning context and was presented with the tone (5 presentations), in the presence of its observer cage-mate (FCbP). The two animals could freely interact throughout this session. The third day, each animal of the triad (FC, FCbP and naïve) was individually tested for fear response to (a) the conditioning context and (b) the tone (in a new context). Naïve mice were the control animals, against which the FCbP mice were compared to assess whether they had learned to fear the tone. Table 1 summarizes the groups we formed to test our conditions, while the Fear Conditioning by-Proxy protocol is presented concisely in Figure 1.

Table 1

<table>
<thead>
<tr>
<th>genotype</th>
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<tr>
<td>ko</td>
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Experimental Design
Figure 1. The Fear Conditioning by-Proxy protocol. On Day 1, the demonstrator of the triad underwent fear conditioning to a tone (FC, black circles). Twenty-four hours later, the FC animal was placed back to the conditioning chamber with its observer cage-mate (FCbP, white circles) and they were presented with the tone. In this session, the FCbP mouse experienced the conditioned responses of the FC animal to the tone. Twenty-four hours later the FCbP mouse was tested for fear expression to the tone.

Apparatus

Each session of the FCbP paradigm was performed in a two-compartment shuttle box [590 (W) x 190 (D) x 240 (H) mm; Panlab, LE 918; Harvard Apparatus Ltd., Holliston, MA, USA] which was enclosed in a second - sound attenuating - box. A fan mounted on the left wall of the sound attenuating box produced white noise and provided ventilation.

The left side of the shuttle box comprised the fear conditioning chamber (conditioning context). Fear conditioning (Day 1), fear conditioning by-proxy (Day 2) and contextual fear test (Day 3) were performed in this context. This chamber was equipped with a stainless steel grid floor connected to a shock generator (Panlab, LE 100-26), a white-light lamp connected to the shuttle box control unit (Panlab, LE 900) and a general sound generator in the back wall that delivered the tone. Operation of the shuttle box (automated tone production and delivery of foot-shocks) used the software program ShutAvoid v.1.8.2. (Harvard Apparatus
In order to test for fear responses to the tone alone (Day 3, cued fear test), we needed to introduce the animals in a context that had not been associated with shocks. The right side of the shuttle box comprised the new context where each animal was tested for fear expression to the tone. Since this side was identical to the left one, we placed white paperboard (a) over the grid floor, (b) diagonally in the chamber in order to alter its dimensions and (c) on the walls’ surface to differentiate them from the black walls of the conditioning context. Furthermore, we decreased the level of illumination in the shuttle box and used 70% acetic acid (instead of ethanol) to clean the chamber between trials.

The position of the mouse in the apparatus was detected by weight transducers located below the grid floor in the two chambers. Behaviour was digitally recorded throughout each session using a camera (Panasonic, CCTV, WV – BP 332EE) mounted on the top of each chamber. All parameters were measured using Ethovision XT8.5 specialized video tracking software.

Procedure

Day 1: Fear Conditioning. The demonstrator of each triad was individually placed in the conditioning chamber and underwent fear conditioning to a tone (in accordance with published methods for classical fear conditioning; Curzon et al., 2009; Caldarone et al., 2000). As shown in Figure 2a, the animal was habituated to the conditioning chamber for 10min and then received five tone-shock pairings: each tone (30s, 1kHz, 85dB) co-terminated with a foot-shock (2s, 0.5mA). The interval between two consecutive tones (inter-trial interval, ITI) was 2min. The FC animal returned to its cage 30s after the last pairing. The animal’s behavior was recorded throughout the test. Afterwards, the observer (FCbP) animal

Ltd). The conditioning chamber was cleaned thoroughly with 70% ethanol before the introduction of each mouse.
of the triad was habituated to the conditioning chamber (10min) and right after placed back to the cage.

**Day 2: Fear Conditioning by-Proxy.** One day after conditioning, the demonstrator and the observer of the triad were placed together in the conditioning chamber for the fear conditioning by-proxy session (Figure 2b). The animals were habituated to the chamber for 10min and then received five tone presentations (no shock, 2min ITI). They could freely interact and their behavior was recorded throughout the session. The animals returned to their cage 30s after the last tone presentation. Subsequently, the naïve control of the triad was separately habituated to the conditioning chamber (10min) and returned to the cage.

**Day 3: Fear learning tests.** Twenty-four hours later, each mouse of the triad was tested for freezing (fear response) to (a) the conditioning context and (b) the tone. For the contextual fear test, each animal was placed individually to the conditioning chamber for 3min and its behavior was recorded. The cued fear test was performed 2h later (Figure 2c). The mouse was placed to a new context and after 3min; it received five tone presentations (30s, 2min ITI). The animal’s behavior was recorded throughout the test.
(a) The fear conditioning session consisted of two phases: pre-tone and tone interval. During the pre-tone interval, the demonstrator was habituated to the conditioning context. In the tone interval, the animal received five tone-shock pairings, with 2min interval between pairings.

(b) The fear conditioning by-proxy session differed from the FC session in two points: the observer and the demonstrator were placed together in the conditioning chamber and there were only five tone presentations (no shock).

(c) Each animal was tested individually for cued fear in a new context. Freezing behavior was recorded in the pre-tone phase (3min) and during the tone phase (5 tones, 2min ITI).
**Behavioural Scoring**

During each test session, we recorded the animals’ behaviour. Two individual raters blind to experimental conditions manually scored *freezing* and *interaction*.

**Freezing.** Freezing constituted the fear index and was defined as the absence of any movement - except breathing - for at least 1s. It was expressed as the percentage of time the animal spend immobile during (1) pre-tone phase [habituation], (2) entire tone phase (tone presentations and ITIs) or (3) tone presentations alone (30s * 5 times; 150s).

**Interaction.** Interaction was expressed as the percentage of time that the observer spent in contact with the demonstrator during (1) the whole session, (2) pre-tone phase/habituation, (3) entire tone phase (tone presentations and ITIs) or (4) tone presentations alone (30s * 5 times; 150s). Social contact was defined as any physical contact or interaction (qualitatively defined below), excluding accidental contact made in passing. This contact comprised of seven unique behaviours that the observer directed towards the demonstrator: allogrooming, paw contact, body contact, sniffing, nose-to-nose contact, play and rattling observation. *Allogrooming* occurred when the observer groomed (licked) the demonstrator. *Paw contact* occurred when the observer placed one or both of his paws on the demonstrator (excluding both accidental contact from trying to get around the demonstrator or using the demonstrator as a support to reach a different area of the chamber). *Body contact* occurred when the observer maintained close contact with the demonstrator by either leaning against the demonstrator or huddling against him. *Sniffing* occurred when the observer actively sniffed at the genital area of the demonstrator. *Nose-to-nose contact* occurred when the two mice touched noses while facing one another. *Play* occurred when the two mice engaged in any mode of playful behavior, including wrestling, pouncing, biting, or chasing. *Rattling observation* occurred when the observer approached and actively observed (stretched toward) the demonstrator’s tail, while the demonstrator displayed tail rattling. Tail rattling constitutes...
an eye-catching fast movement of the tip of the tail, which makes a characteristic sound, and is thought to be a threat behavior (Dennis and John, 1973). Each social contact type was expressed as (1) the number of contacts encountered and (2) the percentage of time the observer displayed the contact, during each interval of interest.

**Statistical Analysis**

The statistical software PASW Statistics 18 (SPSS 18.0) was used for all statistical analyses. The Kolmogorov–Smirnov goodness-of-fit test was used to assess normality (Gaussian-shaped distribution) for all continuous variables. Results are presented as means ± standard errors. For all comparisons, statistical significance was set at p < 0.05.

**Results**

**Day 1: Classical fear conditioning**

During the FC session (Day 1), one mouse of each triad (demonstrator) underwent cued fear conditioning. Figure 3 presents the mean percentage of time each genotype spend freezing during the five consecutive tone-shock pairings. A two-way mixed design ANOVA was performed on these data, in order to examine if β2 knockout mice exhibited differences in cued fear acquisition compared to the wt mice. “Tone-shock pairing” was the within-subject factor (five levels: 1st, 2nd, 3rd, 4th, 5th), “genotype” was the between-subject factor (two levels: β2 ko, wt) and “freezing” was the response variable. As expected, there was a significant main effect of “tone-shock pairing” on freezing duration [Greenhouse-Geisser correction; \(F(3, 61) = 95.33, \ p < .001, \ \eta^2 = .81\)]. Multiple comparisons (Bonferonni correction) revealed that mice froze significantly more during the last three pairings than during the first and the second pairing (\(p < .001\), for each comparison). In addition, mice froze significantly more in the fifth pairing compared to the third one (mean difference:
There was no significant interaction of genotype × tone-shock pairing [Greenhouse-Geisser correction; $F(3, 61) = 0.32, p = .80$], or genotype effect [$F(1, 22) = 0.02, p = .89$] on freezing duration. Thus, no differences were found between knock out and wild type mice in the rate of cued fear learning or the magnitude of fear response (duration of freezing), indicating that $\beta_2$ ko mice exhibited normal acquisition of cued fear.

Figure 3. Fear acquisition to the tone displayed by wt and $\beta_2$ ko demonstrators (Day 1, fear conditioning session). The two genotypes showed no significant difference in the mean percentage of time they spend freezing during each of the five consecutive tone-shock pairings ($p > .05$). In addition, the two genotypes genotypes expressed significant freezing to the tone after the second pairing and froze significantly more in the fifth pairing compared to the third one ($p < .05$, for each comparison). Overall, $\beta_2$ ko mice exhibited normal acquisition of cued fear.
Day 2: Fear conditioning by-proxy

Since the two genotypes displayed no difference in cued fear acquisition, it was expected that during the FCbP session (Day 2) both FC groups would express similar levels of freezing during tone presentation in the presence of their cage-mates (observers). Indeed, no difference was detected between groups (mean difference: 4%, \( p = .65 \); Figure 4a). This finding confirms that young adult β2 knockout mice show unimpaired fear conditioning (Caldarone et al., 2000) and, more importantly, that the same fear information would be available to be transmitted to ko and wt observers about the tone.

In order to examine if the two FC groups adopted the same freezing pattern during the whole FCbP session (pre-tone and tone interval), a two-way mixed design ANOVA was performed on the data of Figure 4b. There were two factors with two levels each: “test-interval” was the within-subject factor (levels: pre-tone, tone), and “genotype” was the between-subject factor (levels: β2 ko, wt). No significant effect of genotype \( [F(1, 19) = 0.36, p = .56] \) or interaction of genotype × test-interval \( [F(1, 19) = 1.43, p = .25] \) was found. There was a significant main effect of “test-interval” on freezing duration \( [F(1, 19) = 23.79, p < .001, \eta^2 = .56] \), suggesting that all FC mice froze significantly more during the tone interval (tone presentation plus ITIs). These findings indicate that β2 ko and wt observers interacted with demonstrators that displayed the same freezing pattern during fear conditioning by-proxy.
Figure 4. (a) Freezing response of wt and β2 ko FC animals to the tone during FCbP session. The two groups displayed similar levels of conditioned fear to the tone, in the presence of the observer (p > .05). (b) Freezing of wt and β2 ko FC animals displayed during each phase of the FCbP session. The two groups froze significantly more during the tone phase (p < 0.01).
Day 3: Testing conditioned fear response

Contextual fear test. The first test on Day 3 assessed the freezing response to the conditioning context. Animals that undergo cued fear conditioning, in subsequent testing express fear not only to the cue, but also – to a lesser extent - to the context in which they had experienced the aversive stimulus (US). Hence, we first examined whether FCbP animals acquired fear to the FCbP context, using a two-way independent measures ANOVA. There were two factors with two levels each: “genotype” (levels: wt, β2 ko) and “group” (levels: FCbP, naïve). A significant main effect of genotype on contextual freezing \( [F(1, 37) = 5.42, p = .03] \) was detected. It is worth to mention though, that both genotypes displayed minimal freezing (< 5%). There was no significant main effect of group on contextual freezing or interaction of genotype × group. Thus, FCbP mice of the two genotypes did not display conditioned fear response to the context. These findings suggest that the fear conditioning by-proxy protocol employed in the present study did not elicit contextual fear.

Cued fear test. In the cued fear test it was examined whether (1) the FCbP animals expressed greater levels of freezing to the tone compared to their naïve controls (fear conditioned response), and (2) there was a genotype effect on fear conditioning by-proxy. For this purpose, a two-way independent measures ANOVA was performed on the data of Figure 5. There were two factors with two levels each: “genotype” (levels: wt, β2 ko) and “group” (levels: FCbP, naïve). There was no significant main effect of genotype and group on tone freezing \( [F(1, 37) = 1.60, p = .21 \text{ and } F(1, 37) = 1.18, p = .29, \text{ respectively}] \) or interaction of genotype × group \( [F(1, 37) = .53, p = .47] \). In other words, neither the wt nor the β2 ko FCbP groups of mice showed significant freezing to the tone.

Although there was no significant mean freezing to the tone for neither genotype, qualitative assessment of the data (Figure 6) indicated that wt FCbP mice displayed greater
Figure 5. Conditioned fear response to the tone displayed by wt and β2 FCBP animals. Wt and β2 FCBP animals displayed similar levels of freezing to the tone (p > .05). Neither group expressed significantly higher freezing to the tone that its respective naïve control group (p > .05).

Figure 6. Histograms of the freezing magnitude (percentage of the total duration of tone presentations) displayed by wt FCBP animals and their naïve controls. FCBP mice displayed greater variation in freezing than naïve controls (FCBP SD = 9.95%, control SD = 2.90%).
variation in freezing than controls (FCbP SD = 9.95%, control SD = 2.90%). One third of the wt FCbP group displayed considerable freezing (>10%; 4 mice), while the rest of the mice did not freeze at all (similar levels with controls; <10%; 8 mice). Interestingly, Bruchey et al. (2010) demonstrated the same effect of a similar FCbP paradigm on rats.

**Exploring potential factors contributing to the differential freezing in the FCbP mice**

*Interaction pattern during FCbP session accounts for freezing variation in the wt observers.* We examined whether (1) the social interaction pattern displayed by the wt observers and/or (2) the conditioned response displayed by their FC cagemate could account for the freezing variation within the wt FCbP group (Table 2). For this purpose, a step-wise multiple regression analysis was performed. The variables shown in Table 2 were the predictors of the wt observers’ freezing response (criterion variable). For wt observers,

Table 2

*Potential factors contributing to the freezing variation displayed by FCbP mice*

<table>
<thead>
<tr>
<th>Social Interaction Pattern</th>
<th>Conditioned Response of FC</th>
</tr>
</thead>
<tbody>
<tr>
<td>interaction during FCbP session (int-t)</td>
<td>freezing during pretone interval (freezing-1)</td>
</tr>
<tr>
<td>interaction during pre-tone interval (int-1)</td>
<td>freezing during tone interval (freezing-2)</td>
</tr>
<tr>
<td>interaction during tone interval (int-2)</td>
<td>freezing-1 minus freezing-2 (freezing-change)</td>
</tr>
<tr>
<td>int-2 minus int-1 (int-change)</td>
<td>freezing during tone (freezing-tone)</td>
</tr>
<tr>
<td>interaction during tone presentations (int-tone)</td>
<td>freezing during ITIs (freezing-ITIs)</td>
</tr>
<tr>
<td>interaction during ITIs (int-ITIs)</td>
<td>Rattling</td>
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<tr>
<td>social contacts</td>
<td>Jump</td>
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<td>nose contact</td>
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<td>rattling observation</td>
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freezing to the tone was significantly correlated with six parameters of social interaction pattern: “interaction change” \(r(10) = .85, p < .001\), “interaction-tone” \(r(10) = .79, p = .001\), “interaction-tone interval” \(r(10) = .73, p = .004\), “interaction-ITIs” \(r(10) = .65, p = .01\), “social contacts-tone” \(r(10) = .61, p = .02\) and “rattling observation-tone” \(r(10) = .72, p = .004\). According to the statistical model (even though the sample was small, all criteria were satisfied: Tolerance > 0.1, VIF < 10, normality of random error, homoscedasticity), only two of the predictors were significant - the percentage of time the observer spent in social interaction with the FC mouse during the tone presentation (interaction during tone), and the change in interaction displayed between the two test-intervals (tone minus pre-tone interaction; interaction change). Both of the predictors were positively correlated with wt observer freezing (Figure 8), and together accounted for 85% of the variance in the group \(R^2 = .85\).

Based on the predictive model, wt observers that enhanced markedly the social interaction in the tone interval (high levels of “interaction change”) and displayed high interaction during tone presentations (high levels of “interaction during tone”) in the FCbP session would be expected to exhibit more freezing to the tone (Day 3: cued fear test) compared to observers with different interaction patterns. As shown in Figure 9, only one third of the wt FCbP group \((\text{FCbP}^+\)) displayed this tone-dependent interaction pattern (high “interaction change” AND high “interaction during tone”), while the rest of the FCbP mice \((\text{FCbP}^-)\) displayed a tone-independent interaction pattern, with quite stable or low interaction during tone (low “interaction change” AND/OR low “interaction during tone”).
Figure 8. Diagrams displaying the correlation between the cued fear of wt observers (Day 3) and the two predictors (Day 2) that accounted for 85% of the freezing variance in the group. Freezing to tone was positively correlated to (a) the time FCbP observers spent in social interaction during the tone presentation ($r = .79$, $p < .001$) and (b) the enhancement of social interaction after the first tone presentation ($r = .85$, $p < .001$) on Day 2.
Figure 9. Diagram displaying the interaction patterns of the wt FCbP mice (FCbP*: tone-dependent interaction, FCbP*: tone-independent interaction) on Day 2. The horizontal line represents the mean interaction change in the FCbP group. Similarly, the vertical line represents the mean interaction during the tone. The mice that enhanced markedly the duration of interaction with the FC mouse during the tone interval and exhibited high levels of interaction during the tone presentation (FCbP+) displayed the higher freezing to the tone on Day 3.

Effect of β2 containing nAChRs on the pattern of social interaction during FCbP. In contrast to wt mice, β2 ko observers displayed similar freezing variation to their naïve controls. In addition, there was no correlation of freezing with any of the factors shown in Table 2. In order to examine for differences between ko and wt observers in social interaction during the FCbP session, we assessed the data in Figure 10. It was demonstrated that during the FCbP session, β2 ko observers spent less time interacting with their FC cagemate than wt
observers did [Student’s t test; \( t (16) = 2.98, p = .009 \)]. Actually, wt observers spent almost the double time in social interaction (11% versus 6% for \( \beta^2 \) ko).

In order to explore further this genotype difference, the duration of social interaction was assessed for each test-interval separately (Figure 11). A two-way mixed design ANOVA was performed on these data: “test-interval” was the within-subject factor (levels: pre-tone, tone), and “genotype” was the between-subject factor. The interaction of genotype \( \times \) test-interval was found significant \([F(1, 19) = 8.53, p = .009, \eta^2 = .31]\). Multivariate analysis of variance (MANOVA) demonstrated that (1) wt observers interacted significantly more than \( \beta^2 \) ko observers during the tone interval \([F(1, 19) = 9.94, p = .005]\), and (2) only wt mice increased their interaction levels in the tone interval \([F(1, 19) = 14.43, p = .001]\).

Interestingly, this interaction enhancement concerned both the tone presentations and the inter-trial intervals [Student’s t test; \( t (11) = -1.80, p = .10 \)]. In other words, knock out mice sustained stable levels of social interaction during FCbP, while wt mice enhanced their interaction levels in the tone interval.

As shown in Figure 12, none of the ko observers displayed tone-dependent interaction (high levels of interaction change in conjunction with high levels of interaction during tone) – the interaction pattern followed by the wt observers with the higher freezing response to the tone. In other words, there were only FCbP mice in the ko group.
Figure 10. The percentage of time that wt and β2 ko observers spent interacting with their respective demonstrator throughout the FCbP session. β2 ko observers interacted less with their FC cage-mate than wt observers did (p < .01, **).

Figure 11. Social interaction displayed by wt and β2 ko observers during each phase of the FCbP session. β2 mice sustained stable levels of interaction throughout FCbP session (p > .05), while wt mice enhanced their interaction levels in the tone interval (p = .005; ***). β2 ko observers interacted significantly less than wt ones during the tone interval (p < 0.01; ##).
Figure 12. Diagram displaying the interaction pattern of the wt and ko FCbP mice (FCbP+: tone-dependent interaction, FCbP−: tone-independent interaction) on Day 2. The horizontal line represents the mean interaction change in the wt FCbP group. Similarly, the vertical line represents the mean interaction during the tone. None of the ko observers displayed the interaction pattern (high levels of interaction change in conjunction with high levels of interaction during tone) demonstrated by the wt observers with the higher freezing response to the tone.

Testing conditioned fear response: FCbP+ and FCbP− wt mice

In order to test whether wt observers with different interaction pattern during the FCbP session displayed significantly different freezing to the tone (cued fear test), we decided to split the wt FCbP group into two subgroups (FCbP+: tone-dependent interaction, FCbP−: tone-independent interaction) and compared these groups to the controls. In more detail, the FCbP+ subgroup displayed interaction change = 13.00 ± 5.48
AND interaction during tone = 20.75 ± 6.70, while the FCbP\(^+\) subgroup displayed interaction change = 2.13 ± 2.70 AND/OR interaction during tone = 8.13 ± 7.57.

The data shown in Figure 7 were analyzed using a two-way mixed design ANOVA: “tone” was the within-subject factor [two levels: pre-tone (new context), tone], and “group” was the between-subject factor (three levels: FCbP\(^+\), FCbP\(^-\), naïve). A significant interaction of tone \times group \[F(2, 20) = 70.93, p = .005, \eta^2 = .41\] was detected, and was further assessed. The effect of group both on pre-tone freezing and on tone freezing were significant \[F(2, 20) = 70.93, p = .005 \text{ and } F(2, 20) = 38.50, p < .001, \text{ respectively}\]. Multiple comparisons (Bonferonni correction; \(a = .0017\)) demonstrated that, although FCbP\(^+\) showed a moderate tendency to freeze more than FCbP\(^-\) in the new context (mean difference: 10\%, \(p = .04\)), both FCbP subgroups displayed similar levels of pre-tone freezing with the controls (\(p > .05\)). This finding suggests that neither subgroup expressed fear to the new context (pre-tone: baseline freezing). During tone, FCbP\(^+\) mice froze more than either FCbP\(^-\) or controls (\(p < .001\), for all comparisons). In addition, the FCbP\(^+\) group froze more to the tone than during the pre-tone phase \[F(1, 20) = 17.49, p < .001\]. These findings suggest that the wt FCbP\(^+\) mice expressed conditioned fear to the tone, while wt FCbP\(^-\) did not. In other words, only wt observers that adopted a tone-dependent interaction pattern displayed fear to the tone.

Overall, these data indicate that (1) a proportion of the wt FCbP mice acquired fear to the tone (wtFCbP\(^+\) versus wtFCbP\(^-\)), while (2) none of the β2 ko FCbP mice expressed conditioned freezing to the tone (koFCbP\(^-\)).
Figure 7. Conditioned fear response to the tone displayed by the two FCbP subgroups (FCbP⁺: tone-dependent interaction, FCbP⁻: tone-independent interaction). The two FCbP subgroups displayed similar levels of pre-tone freezing with the naïve controls ($p > .05$). During tone, FCbP⁺ froze more than FCbP⁻ and naïve controls ($p < .001$, for all comparisons; ###). In addition, FCbP⁺ froze more to the tone than during the pre-tone phase ($p < .001$; ***).

Discussion

Learning to fear cues that predict danger is a major mechanism to shape adaptive behaviours in a constantly changing environment. Learning about potentially harmful stimuli and events from others help humans to adapt rapidly in unfamiliar conditions and, more importantly, in a less risky way than direct experience does. Language comprises the finest evolutionary tool we use in this direction. Nevertheless, we also use nonverbal means to learn from others, namely detection of fear signs. Observational fear learning is a well-documented phenomenon in primates (Hygge and Ohman, 1978; Vaughan and Lanzetta, 1980; Mineka et al., 1984; Mineka and Cook, 1986; Cook and Mineka, 1987; Mineka and Cook, 1993; Olsson
and Phelps, 2004) and developing similar models in rodents would facilitate the study of neural mechanisms underlying indirect fear learning.

Whereas the neural circuitry of fear learning through classical conditioning is understood in considerable detail, researchers have just begun to study the neural mechanisms underlying socially transmitted fear learning. Behavioral findings of observational fear learning in nonhuman animals, followed by research on social fear learning in humans, imply that the basic associative learning processes that are responsible for acquisition and expression of fear are similar across species and across different learning procedures, such as social observation and verbal instruction (Olsson and Phelps, 2007). However, social, affective and cognitive processes are likely to contribute to fear learning in a social context. In the present study, we sought to examine the role of social interaction in observational fear learning and furthermore, to explore a possible involvement of high affinity nAChRs.

Employing the Fear Conditioning by-Proxy paradigm, we managed to demonstrate that mice of the sociable B6 strain can acquire fear to a neutral stimulus, through social interaction with a familiar FC conspecific, in the presence of that stimulus. In other words, a cage-mate’s conditioned response to a tone affected the observer’s subsequent behavior to that tone, but only for some of the wt observers (one third of the FCbP group). The conditioned response (freezing pattern throughout FCbP) displayed by demonstrators during the FCbP session could not account for this freezing variation within the wt FCbP group. To the contrary, two parameters of the social interaction during the FCbP session correlated positively with the freezing displayed by wt observers and together accounted for 85% of the variance in the group. These parameters were: (1) the percentage of time the observer spent in social interaction with the FC mouse during the tone presentations, and (2) the quantitative
change in interaction displayed after the first tone presentation (tone minus pre-tone interaction).

These findings are consistent with those of Bruchey et al. (2010) in rats. Particularly, these researchers found that (1) only half of the FCbP rats displayed freezing to the tone and that (2) the percentage of time the FCbP rats spent in social interaction with the FC animal during the tone (Day 2) significantly correlated with FCbP freezing, accounting for 28.5% of the variance in that group (Day 3). Here we managed to show for the first time, that fear conditioning by-proxy is possible not only in rats (Bruchey et al., 2010), but also in mice; contradicting Kim et al. (2010) who suggested that prior fear experience is required in order for FCbP animals to exhibit freezing to tone. In addition, we extended Bruchey’s findings about the role of social interaction in observational fear learning, by showing that only wt observers that adopted a tone-dependent interaction pattern during observational learning displayed fear response to the tone the next day. In particular, only the wt FCbP subgroup that exhibited markedly enhanced duration of interaction with the FC mouse after the first tone presentation and exhibited high levels of interaction during the tone (Day 2) displayed freezing to the tone (FCbP⁺, Day 3). On the other hand, animals that displayed a tone-independent interaction pattern (FCbP⁻), with quite stable or low interaction during tone, did not freeze to the tone.

Overall, these findings indicate that observational fear learning in wt mice depend on the enhancement of social interaction with the fearful demonstrator during tone. It is quite interesting that FCbP⁺ mice did not express fear to the context of conditioning (contextual fear test, Day 3) although the demonstrator expressed freezing to the context, too (Day 2). The fact that observers were habituated to this context (Day 1) might have helped these mice dissociate it from the conditioned response of the demonstrator, since their prior experience informed them that it was a safe context. In contrast, they were not habituated to the tone.
The first tone presentation induced an enhanced conditioned response of the demonstrator, which lasted throughout the whole tone phase. Accordingly, it seems that either the tone triggered a higher motivation to interact in order to acquire social information about it (enhanced interaction motivated by the tone), or the enhanced freezing of the demonstrator to the tone attracted their attention (enhanced interaction motivated by the conspecific’s conditioned response). In any case, the observers in this group associated the distress of their conspecific with the tone.

There is now evidence suggesting that social interaction correlates with activation in prefrontal and cingulate cortices (Gehring and Knight, 2000). Furthermore, damage in these brain areas has been correlated with impairments in social interaction (human, macaque, and rat studies; Hadland et al., 2003; Scearce-Levie et al., 2008; Rudebeck et al., 2008; Avale et al., 2011). Mice lacking the β2-subunit of nAChRs display morphological alterations in prefrontal cortex (PrL and CC), impaired behavioural flexibility (required for normal social interaction) and increased social interaction (compared with wild type mice) after social isolation. Therefore, β2 knockout mice might be an appropriate animal model to explore further the role of social interaction in observational fear learning, as well as to investigate the underlying neurobiological mechanisms.

In our study, none of the β2 ko observers expressed freezing to the tone (or context), indicating that they failed to acquire fear through social interaction. This result cannot be attributed to impaired fear learning since β2 ko FC mice exhibited normal acquisition and expression of contextual and cued fear, indicating that associative learning and fear memory formation/retrieval were intact in these mice. This finding is consistent with a previous study showing that young adult β2 ko mice (3-4 months old) do not exhibit impaired contextual or tone-conditioned fear (Caldarone et al., 2000). Furthermore, β2 ko FC mice appear to transmit the same fear information (freezing pattern throughout FCbP) during the FCbP
session as wt FC mice did, based on the freezing response of FC mice. It is well known that there is a range of fear responses to a conditioned stimulus. Besides freezing, conditioned animals emit auditory and olfactory signals of fear, such as ultrasonic vocalizations (USVs) and stress-induced anxiogenic pheromone, respectively. Interestingly, it has been suggested that both of these responses to CS affect cued fear learning in observers (Chen et al., 2009; Bredy and Barad, 2009). Nevertheless, a recent study showed that β2 ko mice display normal interest in social olfactory cues such as pheromones (consistent with our data showing no sig. difference in sniffing contacts between observers of the two genotypes), they have no auditory deficits and do not display impaired USVs emission (Chabout et al., 2013). Accordingly, we have no indications that ko observers were exposed to different fear information during FCbP compared to wt mice.

Further exploration of the possible factors contributing to the failure of β2 ko mice to exhibit observational fear learning, revealed that these mice displayed quite stable levels of social interaction with the demonstrators throughout the FCbP session. In other words, all ko FCbP mice displayed tone-independent interaction patterns, similar to wt FCbP animals. This finding further supports the significance of a tone-dependent interaction pattern in FCbP. We cannot attribute this result to impaired motivation for social interaction, since β2 ko mice exhibited normal levels of interaction during the pre-tone phase (consistent with literature; Granon et al., 2003; Chabout et al., 2013). On the contrary, they failed to adapt their interaction to the special conditions during the tone phase, namely they did not increase their interaction, as wt observers did. This finding indicates a possible role of intact β2 nAChRs on normal social interaction - the factor that affected observational fear learning in our study. This impairment in β2 ko mice could be explained by a rigid social behavior (Serreau et al., 2011), an attentional deficit (Howe et al., 2010; Guillem et al., 2011) or a lack of empathy (Preston and de Waal, 2002).
Social behavior is a defining mammalian feature that integrates emotional and motivational processes with external rewarding stimuli. It is thus an appropriate readout for complex behaviors. Social interaction requires sequences of action in which the individual has to adjust rapidly to the unpredicted behavior of others, organize the succession of its actions and inhibit habitual or inappropriate responses. In other words, normal social interaction requires a range of intact executive functions, such as planning complex behaviors, inhibitory response control, decision making and behavioral flexibility. The prefrontal cortex (PFC) is recognized as a crucial brain structure for executive functions. It (1) mediates impulsivity and compulsivity control, and (2) guides behavior by associating sensory processing, memory, and emotions so that the decision-making process allows a flexible and adapted behavior in the face of unexpected outcomes (Dos-Santos Coura and Granon, 2012). PFC is also the site of integration of several neurotransmitter (NT) inputs essential for these processes (Robbins et al., 1998).

There is now evidence that β2-containing AChRs could play a pivotal role in cognition and executive behaviors (Picciotto et al. 1995; Granon et al. 2003; Maubourguet et al. 2009; Avale et al. 2011; Bourgeois et al. 2011) through neurotransmitter release in the PFC (for a review; Dos-Santos Coura and Granon, 2012). As Dos-Santos Coura and Granon (2012) suggested, they are likely to be “crucial for decision-making processes during which there is integration of emotional and motivational features for adapted and flexible goal-directed behaviors”. Data indicating that β2-containing nAChRs are directly implicated in behavioral flexibility (Granon et al., 2003; Serreau et al., 2011; Granon and Changeux, 2012; Chabout et al., 2013) are consistent with our findings on the failure of β2 ko mice to enhance interaction during the special conditions they were exposed to during the tone phase of FCbP. Therefore, we suggest that the rigid social behavior displayed by β2 ko FC mice is the most parsimonious explanation for their failure to acquire cued fear through social observation.
The combination of targeted behavioral research on executive functions in mice, the use of mutant mice and local gene transfer will deepen our knowledge on specific nAChRs functions.

Overall, our results underline the importance of flexible social behaviors in order to acquire information about danger from others. Firstly, we found that mice (of a sociable strain) could exhibit fear behavior to a neutral stimulus after being exposed to the conditioned response of a familiar mouse to that stimulus. Second, the social interaction pattern displayed by observers during observational fear learning, significantly accounted for their subsequent fear response. Particularly, only mice that enhanced interaction with the fearful demonstrator during the stimulus presentation (cue-dependent interaction) acquired fear to the stimulus. Finally, the lack of $\beta_2$-containing AChRs affects the interaction pattern displayed during observational fear learning. Accordingly, it seems that $\beta_2$-containing AChRs affect observational fear learning indirectly, through their effect on social behavior.

Further research is required before we start to unravel the factors that modulate observational fear learning. For instance, it would be interesting to explore the effect of social status in social transfer of fear, as it could affect (1) the interaction between observers and demonstrators and (2) the significance attributed to the social information (Fano et al., 1997; Arregi et al., 2006). Furthermore, prior experience of observers in unpaired shocks could affect the ability of observer mice to acquire observational fear (Kim et al., 2010). If this is the case, then empathy could play an important role in social transfer of fear (Preston and de Waal, 2002; Laland, 2004; Panksepp and Lahvis, 2011). Another interesting approach would be the utilization of the “mother-child” pair as a special model of “demonstrator-observer” in the fear conditioning be-proxy paradigm. This version of observational fear learning could inform us about the ways in which mothers’ emotional responses to neutral stimuli could affect the child’s behavior in later encounters of those stimuli. Depending on the child’s age
and, as a result, on his cognitive development, it could be investigated which cognitive/executive abilities are crucial for social learning. Moreover, important implications could emerge for the acquisition of specific phobias (not preceded by direct experience of a traumatic episode) or more generally, the impact of the mother’s fear responses to their child’s behavior.

In the present study, we utilized freezing as a measure of fear, while there are other indexes that might be more plausible/sensitive to the socially acquired fear. Laxmi et al. (2003), showed that different experimental manipulations (e.g. US intensity and the number of CS and US applied, paired or unpaired presentation of CS and US) can cause a variety of conditioned responses (risk assessment, freezing, flight responses) to the CS with several respective intensities (Laxmi et al., 2003). In other words, there are qualitatively and quantitatively different increases of defensive behavior in response to conditioned stimuli of different salience. During our experiments, we observed a range of risk assessment behaviors (e.g. orientation towards the sound generator, stretched attend, vigilance) from the FCbP mice that could indicate a modest fear expression, anxiety caused by the tone or generally emotional reactivity (Blanchard et al., 2001; Roy and Chapillon, 2004). Therefore, it would be interesting to assess a number of different emotional responses in observers when studying observational fear learning. In this line of thinking, autonomic responses (e.g. skin conductance, blood pressure elevation) would also comprise a useful tool (Delgado et al., 2006). Moreover, the study of ultrasonic vocalizations could help us investigate different ways through which mice acquire fear information socially - as it is now accepted that mice emit ultrasonic vocalizations (USVs) when they are in social contact or in certain emotional states (Panksepp and Lahvis, 2007; Jamain et al., 2008; Scattoni et al., 2008, 2010; Chabout et al., 2012) and that USVs of a conspecific undergoing cued fear conditioning can induce cued fear to a naïve mouse (Chen et al., 2009).
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