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ORIGINAL PAPER

Differing effects of familiarity/kinship in the social transmission of fear associations and food preferences in rats

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Abstract
Despite its apparent adaptive advantages, past research has found that greater familiarity and/or familial relatedness of a social demonstrator does not enhance social learning in the social transmission of food preference paradigm. This finding runs counter to research examining the effects of demonstrator characteristics in fear-mediated social learning paradigms, in which increased familiarity and/or relatedness of a demonstrator promotes higher rates of learning in observer rats. In our first experiment, we were able to corroborate the finding that increased familiarity/relatedness to the demonstrator does not enhance acquisition of a socially transmitted food preference. Furthermore, on examination of the social behavior between observers and their demonstrators during the acquisition of a socially transmitted food preference, no analogous relationship between social contact and expression of the learned preference was observed. In our second experiment, we provide further evidence that familiarity/relatedness may enhance the social acquisition of a fear response to an otherwise neutral auditory cue and demonstrate that this effect is not solely the result of increased social contact between the observer and their demonstrator during acquisition. Despite similar levels of post-cue contact in both observer types, a positive correlation was observed between post-cue social contact and expression of a socially acquired fear behavior when the observer was familiar/related to their demonstrator but not novel/unrelated. These findings both validate previous research on the role of familiarity/relatedness in these two social learning paradigms and provide further behavioral evidence that unique social mechanisms may serve to mediate the social transmission of fear.

Keywords Social learning · Social transmission of food preference · Fear conditioning by-proxy · Familiarity · Social fear learning

Introduction

Social learning, the acquisition of information about the environment through the interaction with or observation of another individual or their biological traces, is a vital source of information for many species. The ability to circumvent the time, effort, and potential risk inherent to learning through direct experience (i.e., asocial learning) provides an adaptive advantage to animals that appropriately utilize social learning strategies. In line with this idea, many nonhuman species have displayed the ability to socially acquire a fear response to cues that signal the presence of imminent threat (Olsson and Phelps 2007), as well as information on food sources in the environment (Galef and Laland 2005). In both cases, asocial learning carries a significant risk to the animal: asocial learning of threat cues risks a direct encounter with said threat, while asocial learning of safe food sources requires energetically costly and dangerous exploration of the environment. While acquiring this information through social means partially eliminates the need for an animal to engage in such risky behaviors, excessive reliance on social learning carries its own risks. As socially learned information is by definition secondhand at best, it does not include potentially crucial information about the context in which it was acquired (e.g., prominent secondary cues or contextual information present in the context of

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acquisition but not present in the context of demonstration). Without this contextual knowledge, there is increased chance that an animal will inappropriately act on the acquired information. Furthermore, theoretical models that have examined social learning on a population-level scale have consistently found that, in a changing environment, indiscriminate social learning is actively maladaptive (Rendell et al. 2011; Kendal et al. 2018). Where asocial learning gives individuals the opportunity to closely sample the environment and update their behavior accordingly, social learning relies on behaviors that may have been acquired in an older version of the environment and, as such, may no longer be adaptive. This allows for behavioral adaption in the event that changes in the environment render old behaviors less effective or maladaptive.

One way that the potential consequences of overreliance on social learning may be mitigated is through increased selectivity on the part of the observer in deciding from whom to learn. Species that rely primarily on information obtained from conspecifics that are more likely to transmit relevant and reliable information should experience greater success at an individual level and, at a population level, should exhibit a balance between reliance on social and asocial learning. Familiarity and relatedness are two strong indicators that a conspecific is likely to carry useful information. Familiar/related conspecifics tend to share biological characteristics with and/or inhabit the same environment as the learner, making the information they carry more likely to be relevant and up to date.

We previously demonstrated this particular learning bias using the fear conditioning by-proxy (FCbP) social learning paradigm in rats (Jones et al. 2014). In FCbP, a naïve rat (the fear-conditioned by-proxy [FCbP]/observer rat; from here on referred to as the *observer*) is allowed to interact with a fear-conditioned conspecific (the fear-conditioned [FC]/demonstrator rat; from here on referred to as the *demonstrator*), while a feared stimulus [conditioned stimulus (CS); an auditory cue that demonstrators had previously experienced as co-terminating with a foot-shock] is presented. Under certain conditions, observers will subsequently respond to the presentation of the CS with fear-induced freezing behavior, even when it is played without the demonstrator present (Jones et al. 2014, 2018; Bruchey et al. 2010; Jones and Monfils 2016a, b). This response can be taken to indicate that observers—despite having no first-hand aversive experiences associated with the cue—have developed a fear association to the CS. Observers whose demonstrators are highly familiar/related to them show increased social fear learning (Jones et al. 2014). The enhancing effect of demonstrator familiarity has also been found in similar fear-based models of social learning in rodents (Kavaliers et al. 2005; Jeon et al. 2010), providing further evidence that rodents modulate their fear learning based on demonstrator familiarity.

The effect of demonstrator familiarity/relatedness has also been examined in the social transmission of food preference (STFP) paradigm. In the STFP paradigm, a food-deprived rat (the demonstrator in this paradigm) is allowed to consume a novel food, after which they interact with a naïve rat (again the observer) (Galef 2003). Following this interaction, the observer is given the chance to eat either the demonstrated food or a novel food. Almost invariably, observers will display a strong preference for the demonstrated food (Galef et al. 1984), an indication that social learning has occurred. However, there is no indication that observers acquire a socially transmitted food preference any better from a familiar or related demonstrator than from a novel demonstrator (Galef and Whiskin 2008). In fact, observers display a preference for food eaten by the novel demonstrator over that of a food eaten by a familiar demonstrator (Galef and Whiskin 2008).

The inconsistent effect of demonstrator familiarity/relatedness on social learning between FCbP and STFP may be partially explained by differing patterns of social contact during acquisition based on the observer's familiarity with and/or genetic similarity to the demonstrator. Jones et al. (2014) were able to demonstrate that, in the FCbP paradigm, observers with a sister demonstrator that they had been housed with since weaning displayed increased social contact immediately following the CS presentation as compared to observers with unrelated demonstrators that they had been housed with for 1 week (1 week familiar). In both conditions, a significant positive correlation was identified between the amount of time which the observer spent socially contacting their demonstrator immediately post-CS presentation and their freezing to the cue at a long-term memory test. However, the results presented by Jones et al. (2014) did not test social fear transmission between an observer and an entirely novel demonstrator. In one experiment using the STFP paradigm, Galef and Whiskin (2008) placed observers in the central compartment of an interaction chamber with access to two demonstrators, one familiar and one novel. Demonstrators were housed in smaller chambers located on either side of the cage and separated from the observer by a wire mesh partition. Galef and Whiskin found that observers tended to spend more time oriented towards the novel demonstrator. Subsequently, observers preferred the food eaten by the novel demonstrator, possibly due to increased exposure to food odor/CS_2 combination. In FCbP, however, it has been difficult to pin down the exact role that social contact plays in the transmission of information between the demonstrator and observer.

We conducted a set of experiments aimed at validating the results of these studies and more closely examining the role that social contact plays in the transmission of information between conspecifics. In our first experiment, we compared the food consumption patterns of rats with related and familiar demonstrators to rats with novel and unrelated demonstrators in the STFP paradigm. In a second experiment, we sought to clarify the findings of our earlier research in FCbP by comparing fear behavior of rats that had observed a familiar and related cage-mate respond to a fear-conditioned cue to rats that had observed a novel and unrelated rat in the same conditions. This experiment aimed to determine whether rats would also display reduced freezing if the demonstrator rat was entirely novel to them (i.e., a stranger). As novel conspecifics demand more attention than familiar rats (Mathiasen and DiCamillo 2010), this would help to determine whether increased examination of a novel and unrelated demonstrator alone is enough to promote social acquisition of a fear association to the levels found in observers with familiar and related demonstrators. In both experiments, the social behavior during the acquisition phase of learning was analyzed and compared between the two observer conditions.

Experiment 1: social transmission of food preference in sisters vs. strangers

Materials and methods

Subjects

Subjects were 33 female Sprague–Dawley rats, aged between 87 and 93 days. All rats were bred in the University of Texas at Austin's Animal Resource Center and were weaned into the same-sex triads of siblings at 21 days of age. Eight breeding pairs were used and consisted of female Sprague–Dawleys (215–260 g) obtained from Charles-River and male Sprague–Dawleys (275–300 g) obtained from Envigo (Houston, TX, USA). Female pups were retained for use in this experiment, while male pups were used in separate experiments.

Diets

We prepared two novel diets, diet cinnamon (Cin) and diet cocoa (Co), by mixing 100 g of powdered 5LL2 Purina rodent chow with either 1 g of McCormick ground cinnamon (diet Cin) or 2 g Hershey cocoa powder (diet Co).

Apparatus

Rats were housed in clear plastic cages and placed on a 12 h light/dark cycle (lights on at 3 am). All phases of the experiment took place in standard rat cages (10.5″ × 19″ × 8″) with the exception of the interaction phase, which took place in a large plastic bin (19.875″ × 15.5″ × 14.75″) with woodchip bedding covering the bottom. Bedding was refreshed between groups. Novel foods were presented to rats in hanging feeders constructed from 4 oz. hexagonal glass jars and 12-gauge steel utility wire. Behavioral procedures were performed under red light during the rats' dark cycle.

Procedure

Each rat in a triad was randomly assigned to one of the three conditions: Demonstrator (Dem), Observer with Familiar Related Demonstrator (Obs-FR), or Observer with Novel Unrelated Demonstrator (Obs-NU) (See Fig. 1). Each triad of sisters was then paired off with another triad consisting of rats born to a separate breeding pair. The demonstrator in each paired cage would serve as the demonstrator for the other cage's Obs-NU rat. Once all group assignments were made, the experiment proceeded as follows:

- *Food deprivation and separation (day 1)*: All demonstrators were moved to single housing during their dark cycle and food deprived for 24 h. During food deprivation, demonstrators were allowed ad libitum access to water. Observer rats remained housed together in their home cage with ad libitum food access.
- *Interaction/acquisition phase (day 2)*: Following food deprivation, demonstrators were transported to an adjacent room and moved to a fresh cage. Hanging feeders containing 30 g of diet Cin were placed in the cage and demonstrators were allowed ad libitum access for 1 h. Once demonstrators had finished eating, the feeder was removed and the remaining food was weighed. Demonstrators were then placed in an interaction bin and allowed to interact with their paired Obs-FR rat and their paired Obs-NU rat. Each interaction occurred independently and lasted for 30 min. The order in which demonstrators interacted with observers was counterbalanced, so that roughly half of the demonstrators interacted first with their Obs-FR, while the other half interacted first with their Obs-NU. Interaction sessions were videotaped for later behavioral scoring (see data scoring and analysis). Following these interactions, demonstrators were returned to single housing and observers were returned to pair housing. Feeders were removed and all rats were food deprived for 24 h.
- *Recall phase (day 3):* Following food deprivation, all rats were moved to an adjacent room and placed alone in a standard housing cage. Each cage contained two feeders hung on opposite ends of the cage, one containing 30 g of diet Cin, the demonstrated diet, and the other containing 30 g of diet Co, a novel diet. All rats were given 1 h to eat before being removed from the cage and returned to their original triads. Remaining food was weighed to determine how much of a given diet each rat had eaten. Demonstrators were run through this choice test as well to gauge whether our population of rats might have an innate preference for either diet.

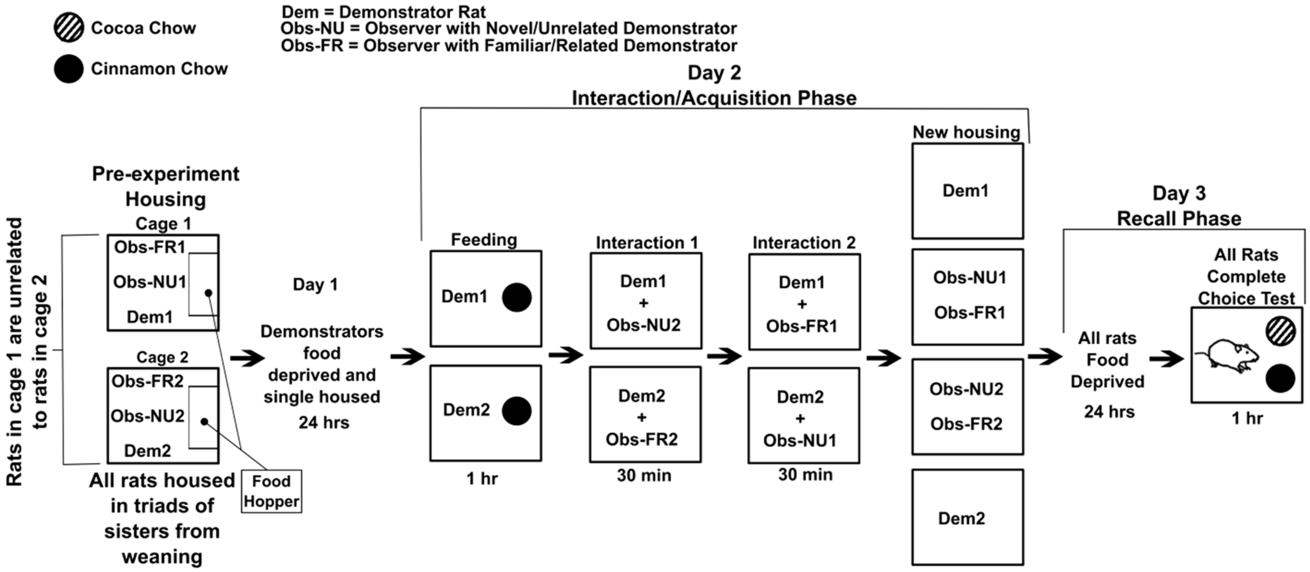

Fig. 1 Experiment 1 design—familiar vs novel demonstrators in the STFP paradigm. All rats were housed in triads with two sisters from weaning. Each rat in a given triad was assigned to either the demonstrator, observer with familiar demonstrator (Obs-FR), or observer with novel demonstrator (Obs-NU) condition. Each triad was paired with a triad of rats that were unrelated to them. On day 1 of the experiment, all demonstrators were moved to single housing and food deprived. On day 2, demonstrators were allowed 1 h of access to cinnamon flavored food and subsequently allowed to interact with the rat from their triad assigned to the Obs-FR condition and the rat from their paired triad assigned to the Obs-NU condition. The order of interaction was counterbalanced. Following these interactions, demonstrators were returned to single housing and all rats were food deprived for 24 h. On day 3, all rats were allowed simultaneous access to pre-weighed containers of cinnamon and cocoa chow. Following the end of the choice test, the remaining food was weighed and the amount eaten for each rat was calculated to determine whether a preference was displayed for either flavor

Data scoring and analysis

Social behavior

The total duration of all social behaviors was scored for during first 10 min of the interaction period on day 2. All social behaviors were scored and general social contact was calculated as described in Jones et al. (2014) with the exception of the "sniffing" behavior, which was divided into "face sniffing" and "body sniffing." "Nose-to-nose contact" and "face sniffing" were also scored during the last 20 min of the interaction period. This allowed us to gauge whether there was an overall difference in the degree of exposure to the combined food and CS_2 scent between observer conditions.

Results

Except where specified, analyses were run on the percent total eaten of the demonstrated diet (Cin), calculated using the following formula:

$$D_{\mathrm{Cin}}/(D_{\mathrm{Cin}} + D_{\mathrm{Co}}) \times 100; D_{\mathrm{Diet}}$$
= the total grams eaten of diet n at the choice test.

Four observers were not included in the final analyses: two because their demonstrator failed to eat prior to the interaction phase on day 2 and two due to a failure to eat during the choice test. Also, as a result of a recording failure, one of the observers included in the choice test analyses was not included in the interaction analyses.

Choice test

A one-way ANOVA with percent demonstrated diet (Cin) eaten as the dependent variable found a significant overall effect of condition (Obs-FR, Obs-NU, or Demonstrator) ($F_{2,26} = 4.697$, $p = 0.018$). A post hoc analysis using Tukey's HSD found a significant difference between the percent of total eaten of cinnamon chow by rats in the Demonstrator condition (M=56.2, SD = 26.35) and rats in the Obs-NU condition (M = 82.5, SD = 7.57) (p = 0.015), while no significant difference between either of these groups and rats in the Obs-FR condition (M= 71.59, SD=14.39) (all ps > 0.1) (Fig. 2a). A two-tailed independent t test verified that observers that were the first to interact with their demonstrator did not show a significant difference in the percent total eaten of the demonstrated diet ($t_{16} = 0.66$, $p > 0.1$; Fig. 2b). Finally, a one-way ANOVA was run to determine whether experimental condition influenced the total amount of food in grams that was eaten during the choice test. Results

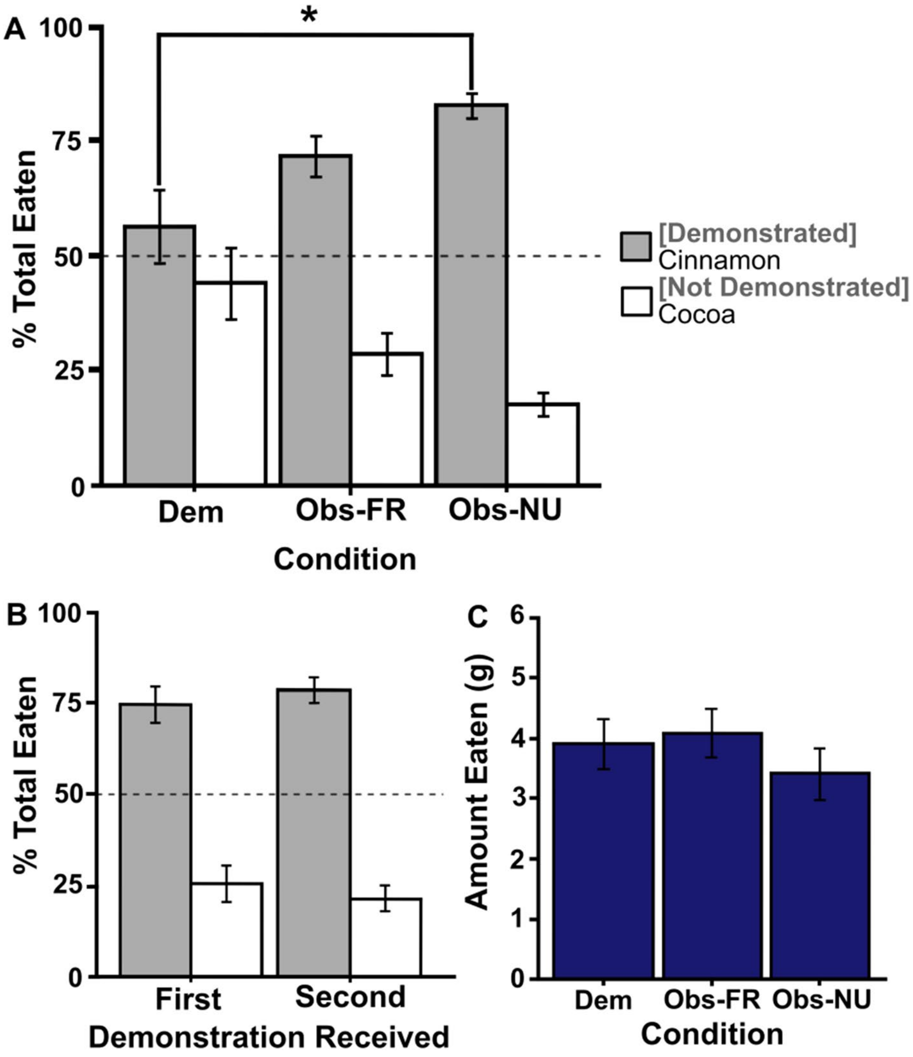

Fig. 2 Food consumption during choice test. **a** The percent of total chow eaten of the cinnamon and cocoa flavors at the choice test by demonstrators (Dem), observers with a familiar demonstrator (Obs-FR), and observers with a novel demonstrator (Obs-NU). Rats in the Obs-NU condition ate significantly more of the demonstrated diet than rats in the Dem condition. **b** The percent of total chow eaten of the two diets in observers based on whether an observer was the first to interact with their demonstrator or the second. **c** The total amount eaten in grams during the choice test ($^*p < 0.05$)

indicated that no such effect was present ($F_{2,26} = 0.613$, $p > 0.1$, Fig. 2c).

Social behavior

Two-tailed independent *t* tests were run to determine whether there was a significant difference in the percent of the interaction period during which Obs-NU rats and Obs-FR rats engaged in nose-to-nose contact, face sniffing, or general social contact. The duration of the nose-to-nose contact and face sniffing was calculated from the full 30-min interaction, while overall social contact was only scored for the first 10 min of the interaction. Due to various violations of *t* test assumptions/outliers, all data were log transformed. The data were plotted using the untransformed results for better readability. While no significant difference was detected between the groups in the total duration of either nose contact ($t_{15} = 0.989$, $p > 0.1$) or the total duration of face sniffing ($t_{15} = 1.88$, $p = 0.08$) (Fig. 3b), Obs-NU rats did display an overall increase in social contact directed towards their demonstrator during the first 10 min of the interaction period ($t_{15} = 3.19$, $p < 0.01$) (Fig. 3a). A linear model was run using the percent of total eaten made up by the demonstrated food as the dependent variable and the percent duration of general social contact, demonstrator type, and duration of face sniffing as independent variables. Interactions between demonstrator type and any of the other independent variables were also tested. Due to high collinearity between the nose-to-nose contact and face sniffing measures, a separate linear model was run to test the predictive value of the percent time spent in nose-to-nose contact. None of the independent variables in either model were found to be significantly predictive of the percent consumption of the demonstrated food (all ps > 0.05, general social contact displayed in Fig. 3c).

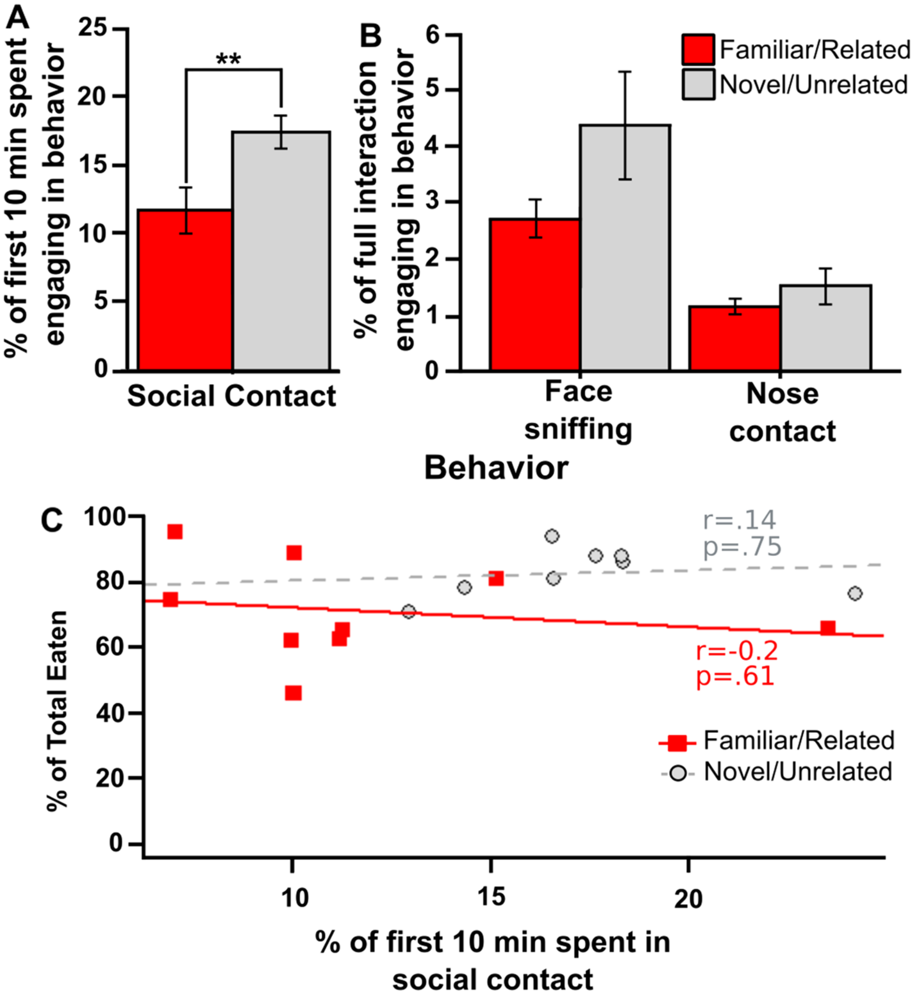

Fig. 3 Social contact during interaction. **a** The percent of time during the first 10 min of the interaction period during which observers were engaged in some form of social contact with their demonstrator. Observers with novel demonstrators spent significantly more time in social contact with their demonstrator than observers with familiar demonstrators (**$p < 0.01$). **b** The percent of time during the full 30 min interaction that observers spent sniffing the face of their demonstrator or in direct nose contact with their demonstrator. No significant differences were present. **c** The percent of total eaten made up by the demonstrated diet as predicted by the percent of time during the first 10 min during which an observer was in social contact with their demonstrator. The relationship was nonsignificant for both observers with novel demonstrators ($r = 0.14$, $p > 0.7$) and observers with familiar demonstrators ($r = -0.2$, $p > 0.6$)

Experiment 2: fear conditioning by-proxy in sisters vs. strangers

Materials and methods

Subjects

Male and female Sprague–Dawley rats (215–350 g) obtained from Harlan (Houston, TX, USA; Harlan now known as Envigo) were used for breeding at The University of Texas at Austin (see (Jones et al. 2014; Jones and Monfils 2016a, b) for details). Rats were housed in light (12:12 light:dark cycle, lights on at 7 am), temperature, and humidity-controlled conditions. Pups were weaned at 21 days of age into triads of the same-sex littermates and remained undisturbed (with the exception of routine animal husbandry) until adulthood (average age at behavioral testing = 85 days). 132 of the female offspring that resulted were used in this experiment and male offspring were used in other experiments (see Jones and Monfils 2016a, b). Food (standard rat chow) and water were provided ad libitum. All procedures were conducted in compliance with the National Institutes of Health Guide for the Care and Use of Experimental Animals and were approved by the University of Texas at Austin Animal Care and Use Committee.

Apparatus

Behavioral procedures took place in standard conditioning chambers during the first half of the light cycle. Chambers were equipped with two metal walls, two clear plexiglass walls, and stainless-steel rod floors connected to a shock generator (Coulbourn Instruments, Allentown, PA), and were enclosed in acoustic isolation boxes (Coulbourn Instruments) and lit with a red light. Behavior was recorded with digital cameras mounted on the top of each unit. The chambers were wiped with soap and water between each session. Stimulus delivery was controlled using Freeze Frame software (Coulbourn Instruments). In each experiment, the conditioned stimuli used in the fear conditioning by-proxy paradigm was a tone (5 kHz, 80 dB), 20 s in duration, and the unconditioned stimulus was a 0.7 mA foot-shock, 500 ms in duration.

Procedure

Rats were housed in triads with the siblings which they were weaned with (see Fig. 4 for visualization of procedure). Each triad consisted of one rat to be fear-conditioned [FC/Demonstrator (FC/Dem)], one rat to be fear-conditioned by-proxy [FCbP/Observer (FCbP/Obs)], and one rat that would not be conditioned (No FC control). Half of the triads underwent the FCbP paradigm (described below) with related cage-mates [observers with familiar/related (FR) demonstrators; FCbP/Obs-FR condition] and the other half underwent FCbP with novel and unrelated conspecifics [observers with novel/unrelated (NU) demonstrators; FCbP/Obs-NU condition]. As such, each cage was assigned to either familiar/related or novel/unrelated conditions and each individual rat within a cage was assigned to either FC/Dem, FCbP/Obs, or No FC control conditions. This design results in each cage containing a No FC housing control rat who is not exposed to a demonstrator within the fear conditioning context, but is still housed with both a demonstrator and observer. Cages assigned to the FCbP/Obs-NU condition were separated in the colony by at least one cage, but remained cage-mates with littermates. NU observer–demonstrator pairs interacted with each other for the first, and only, time in the fear conditioning chamber at the start of the FCbP procedure on day 2. The FCbP procedure was as follows, with 24 h between testing days:

- *Fear conditioning (demonstrator FC; day 1):* one rat in each triad was assigned to the FC/Dem condition. On the first day of experimentation, this rat was removed from the home cage and placed (alone) into a fear conditioning chamber. After a 10 min habituation period, this rat received three presentations of the CS [inter-trial interval (ITI) = 180 s on average, variable], each co-terminating with an aversive foot-shock (0.7 mA, 500 ms). At the termination of the fear conditioning procedure, the FC/Dem rat was returned to her home cage.
- *Fear conditioning by-proxy (social acquisition—FCbP; day 2)*: The FC/Dem rat was returned to the fear conditioning chamber accompanied by a previously naïve FCbP/Obs rat (either a cage-mate or a naïve animal from a different cage). The CS was presented three times (vari-

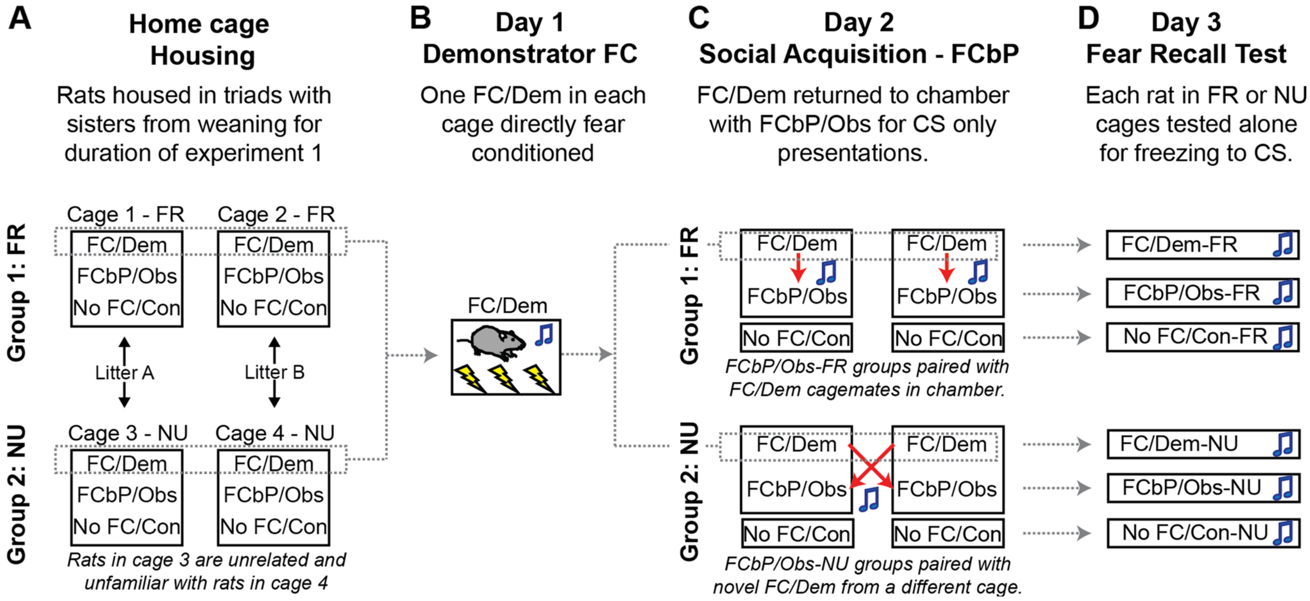

Fig. 4 Experiment design for experiment 1—fear conditioning by-proxy paradigm in Familiar/Related and Novel/Unrelated rats. **a** Example of home cage housing conditions of four sample cages. Cages 1 and 2 were assigned to Familiar/Related group (FR) and cages 3 and 4 were assigned to Novel/Unrelated groups (NU). Female rats were housed from weaning in triads with the same-sex litter mates. One rat per cage was assigned to either FC/Demonstrator, FCbP/Observer, or No FC/Control conditions, with all three conditions in each cage. Immediately after testing on each day, rats were returned to these original housing conditions. **b** On day 1, the FC/Dem rat was directly fear-conditioned. This rat was placed in a chamber alone and received three presentations of an auditory conditioned stimulus (CS) co-terminating with a foot-shock (US). **c** On day 2, FC/Dem rats were placed in the fear conditioning context with either a FR cage-mate (FCbP/Obs-FR assigned rat) or with a Novel/Unrelated rat from a different home cage (FCbP/Obs-NU assigned rat). Three CS presentations were played, while both rats were in the chamber. Arrows indicate Dem–Obs pairs in chamber relative to housing assignments. Third, No FC rat remained in the original home cage as a housing control, while Dem–Obs pairs underwent fear conditioning by-proxy (either as FR or NU pairs). **d** On day 3, each rat was tested for fear recall alone in the chamber with three presentations of the CS

able ITI, mean = 180 s). No foot-shock was delivered. The third rat of each triad (No FC) remained in the home cage. At the termination of the FCbP procedure, FC/Dem and FCbP/Obs rats were returned to their respective home cages.

- *Long-term memory—fear recall test (LTM; day 3)*: Each rat (FC/Dem, FCbP/Obs, and No FC) was placed in the fear conditioning chambers alone and received a long-term memory test (three CS presentations; variable ITI, mean = 180 s) to assess fear expression to the tone. No foot-shock was delivered. Each home cage contained one FC/Dem rat, one FCbP/Obs rat, and one No FC rat.

Data scoring and analysis

Freezing

Videos were scored for freezing behavior both during presentation of the CS, as a measure of cued fear, and for the 20 s immediately preceding each CS, as a measure of contextual fear. Freezing was defined as the absence of any movement, excluding breathing and whisker twitching. The total number of seconds spent freezing throughout the CS presentation is expressed as a percentage of CS duration (20 s).

Social contact

Social contact was quantified on day 2, when both an observer and demonstrator were present in the fear conditioning chamber. Social contact was defined as any physical contact or interaction between the observer and demonstrator rat, excluding accidental contact made in passing, measured as the percentage of time engaged in social interactions for the first minute (when first placed in the chamber), throughout the duration of each CS, and during the 20 s following each CS presentation. The first minute was chosen to sample social interactions when first placed in the new environment, and was when the majority of social interactions occurred prior to CS presentation. The following social interaction types were quantified: 1) direct body contact, including paw-to-body or body-to-body contact, 2) approaching and investigating, including snout orientation within 1 cm of the other rat and sniffing, 3) grooming, including allogrooming of the face and body as well as anogenital grooming (see Bruchey et al. (2010) for details). Aggressive and play behaviors did not occur, while the rats were in the chamber.

Results

Consistent with previous applications of the FCbP paradigm (Jones et al. 2014; Bruchey et al. 2010; Jones and Monfils 2016a, b), freezing displayed by FCbP/Obs rats with a familiar demonstrator during CS presentations on day 3 was widely distributed ($SD_{familiar} = 0.12$, $SD_{novel} = 0.03$). Freezing in the FCbP/Obs rats did not meet the assumption of equal variances (Levene's $F_{42} = 15.7$, $p < 0.001$) and degrees of freedom for analysis were adjusted accordingly. As a group, the FCbP/Obs-FR rats froze significantly more than the FCbP/Obs-NU rats during long-term memory tests on day 3 ($t_{26} = 3.24$, $p = 0.003$) (Fig. 5b) despite no differences in freezing during CS presentations on day 2 ($t_{42} = 0.36$, $p > 0.05$) (Fig. 5a). There was essentially no freezing displayed (< 5%) by the No FC rat of the triad when presented with the cues on day 3, and the social relationship between the FC/Dem and FCbP/Obs rat on day 2 did not influence freezing in the No FC rat, when first presented with the CS ($t_{42} = 0.03$, $p > 0.05$) (Fig. 5b). In addition, contextual freezing was minimal prior to CS presentation and no different between FR and NU groups (Table 1). Consistent with what we present here, we have previously found high variability in this paradigm and hypothesize that the social interactions that occur between the observer and demonstrators may account for some of this variation. Given the nature of this design and the emphasis given to the extent of social interactions between rats, social interactions between all rats were measured for the first 1 min that the rats were placed in the conditioning chamber together. Independent sample *t* tests were performed on percent social contact displayed during both the first minute of pairing in day 2 as well as the average percent of time spent engaged in social contact during the 20 s following each cue. Homogeneity of variance assumptions was not met for social contact occurring during the first minute ($SD_{familiar} = 0.03$; $SD_{novel} = 0.07$) (Levene's $F_{42} = 9.42$, $p = < 0.01$) and degrees of freedom were adjusted accordingly. Novel/unrelated pairs spent a significantly greater percentage of time engaged in social interactions than familiar/related pairs during the first minute of pairing on day 2 ($t_{28} = 2.6$, $p = 0.014$) (Fig. 6a). In contrast with the results from Jones et al. (2014) between pairs that were both related and cage-mates since weaning and pairs that were unrelated and only 1 week familiar, there were no quantitative differences in the amount of time spent engaged in social contact immediately following termination of the cue between familiar/related pairs and novel/unrelated pairs ($t_{42} = 0.11$, $p = 0.91$) (Fig. 6a). Linear regressions revealed that, despite the social interactions that occur between the novel/unrelated rats, there was only a positive linear relationship between post-cue social contact between the FC/Dem and FCbP/Obs rats on day 2 and freezing displayed by the FCbP/Obs rat on day 3 if the FC/Dem and FCbP/Obs pair in the chamber were familiar/related, $R_{23} = 0.58$, $p = 0.004$ (novel/unrelated pairs: $R_{21} = 0.19$, $p = 0.42$ (Fig. 6b). This relationship was driven by direct body contact with the FCbP animal (either body-to-body or paw-to-body) initiated by the FC rat (linear regression model ANOVA $F(6,15) = 7.617$,

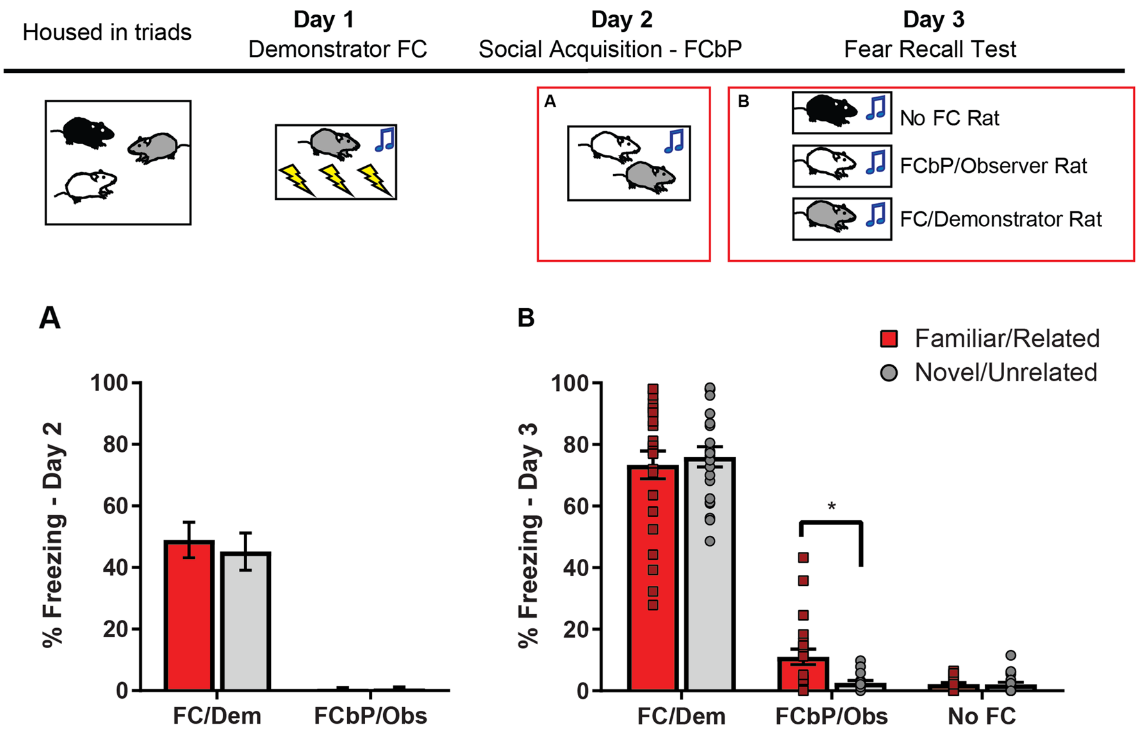

Fig. 5 Cued Freezing in triads with Familiar demonstrators and Novel demonstrators. **a** Freezing to the cues in FC/Demonstrator and FCbP/Observer rats on day 2. **b** FCbP/Obs-FR rats (n =23) froze more to the conditioned stimulus on day 3, after social fear acquisition with a Familiar/Related cage-mate than FCbP/Obs-NU rats ($n=21$) paired with a Novel/Unfamiliar conspecific (*$p<0.05$)

Table 1 Contextual freezing in all rats on day 3 was minimal

	Familiar/related			Novel/unrelated		
	Mean (%)	SD (%)	*n*	Mean (%)	SD (%)	*n*
FC/Dem	17.19	18.08	23	20.32	20.04	21
FCbP/Obs	0.81	1.67	23	1.06	1.52	21
No FC/Con	0.59	1.18	23	0.55	1.37	21

Contextual freezing was measured 20 s immediately prior to each CS presentation and averaged across all three CSs for each rat. Neither FCbP/Obs nor No FC/Con rats display freezing to the context prior to CS presentation. Rats that served as demonstrators, and received directly CS+US pairings 48 h prior displayed ~ 20% freezing in both groups

$p=0.004$; $R=0.914$, $R^2=0.835$; see coefficients in Table 2), with increased total duration of direct body contact initiated by the FC rat towards the FCbP after CS termination predicting increased social fear learning in the FCbP rat. This relationship was not present in the stranger rats (all p values > 0.475; model ANOVA $F(6,14)=0.184$, $p=0.973$).

We further subdivided social contact by both directionality (e.g., FC/Dem initiates contact towards FCbP/Obs or FCbP/Obs initiates contact towards the FC/Dem rat) and types of contact described above. There was a significant increase in the proportion of social contacts initiated by the FCbP rat in both groups after CS presentation compared to the first 1 min in the chamber when both FCbP and FC rats initiate equal amounts of social interactions (Wilcoxon Signed Rank Test $p=0.044$) (Fig. 7a). This could be due to residual freezing from the FC rat that limits contact initiation. Regardless of familiarity group, FCbP rats initiated more contact in the 20 s immediately following CS presentation than the FC rats (paired t test $t_{30}=2.764$, $p=0.010$) (Fig. 7a). There were no group differences between the likelihood to engage in a specific subtype of social behavior during any of the timepoints sampled in the chamber (all p values > 0.2).

In agreement with our previous applications of the FCbP paradigm (Jones et al. 2014; Bruchey et al. 2010), freezing displayed by the FC/Dem rat to the CS during the FCbP session on day 2 was not correlated with freezing displayed by the FCbP/Obs rat the following day in rats paired with

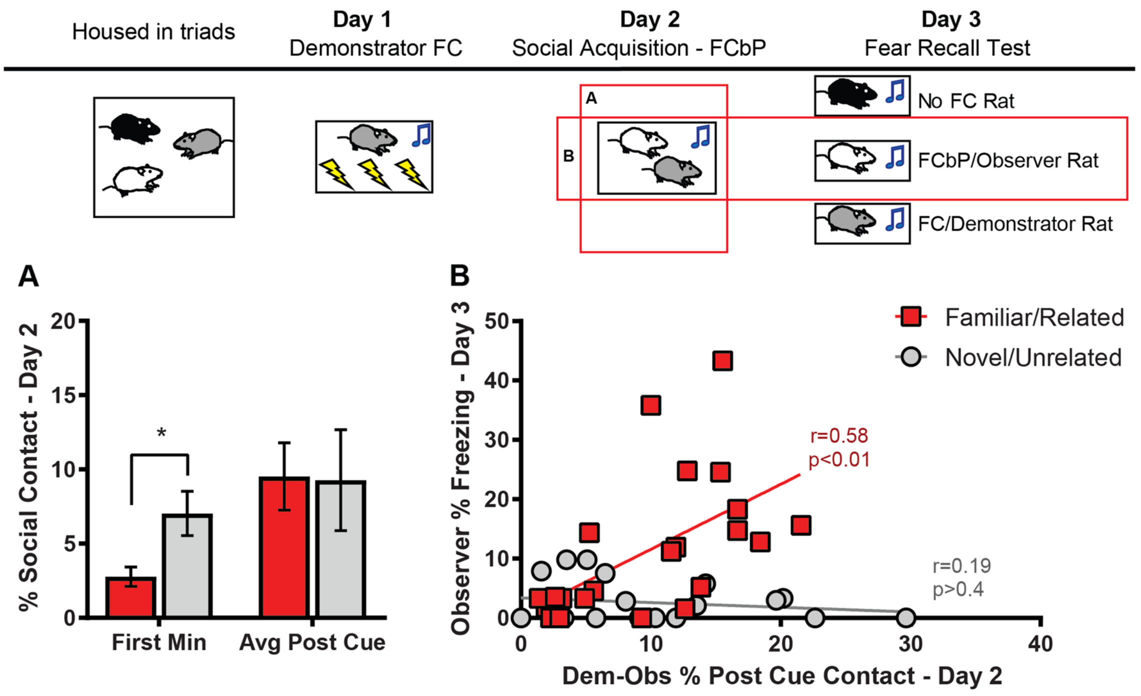

Fig. 6 Social contact between FCbP/Observer and FC/Demonstrator rats. **a** FCbP/Observer rats with a novel/unrelated FC/Demonstrator rats displayed more social contact during the first minute of pairing on day 2 than familiar/related pairs ($p = 0.014$) and there were no differences in social contact occurring in the 20 s immediately following cue termination. **b** Despite equal amounts of post-cue contact on day 2, this contact only predicted day 3 freezing in FCbP/Observer rats paired with a familiar/related FC/Demonstrator ($R = 0.58$; $p < 0.01$)

a familiar/related FC/Dem ($R_{23} = 0.2$, $p > 0.05$) or rats with novel/unrelated FC/Dem ($R_{21} = 0.3$, $p > 0.05$) (Fig. 8). A repeated samples *t* test revealed that rats did, however, freeze less to the CS in the presence of a conspecific (day 2) than they did when tested alone the following day ($t_{43} = 9.46$, $p < 0.001$) regardless of familiarity group and independent samples t test showed that there are no familiarity group differences in freezing displayed by the FC/Dem rat after direct fear conditioning on day 2 ($t_{42} = 0.5$, $p > 0.05$) or day 3 ($t_{42} = 0.46$, $p > 0.05$).

Table 2 Linear regression coefficients of social contact subtypes and directionality

Predictor	Beta	*p*
FC initiates—body contact	.483	0.013
FC initiates—approach	.137	0.503
FC initiates—grooming	.412	0.118
FCbP initiates—body contact	.244	0.291
FCbP initiates—approach	.017	0.932
FCbP initiates—grooming	.307	0.169

Results were totaled from the 20 s immediately post-cue on day 2 on day 3 freezing in Familiar/Related rats. Although the demonstrator rats initiated social contact significantly less than the observer rats after CS presentation, when they did initiate direct body contact with the observer, this was the strongest predictor of observer freezing the following day

Discussion

The results of our experiments indicate that the effect of demonstrator familiarity on acquisition of information through social learning is dependent on the type of information being transmitted. In agreement with previous research on STFP (Galef and Whiskin 2008), observers that underwent STFP with familiar/related demonstrators did not socially acquire a stronger taste preference. In contrast, observers in the FCbP paradigm that had received their demonstration from a familiar/related rat displayed higher freezing in response to a socially conditioned stimulus than subjects observing a novel conspecific. Our results in FCbP are consistent with previous findings out of our own (Jones et al. 2014) and other labs (Kavaliers et al. 2005; Jeon et al. 2010) that have examined the effect of familiarity/kinship on social fear transmission.

We also found that despite similar amounts of post-cue social contact, FCbP observers with familiar/related demonstrators displayed significantly higher freezing in response to the cue at the long-term memory test. Furthermore, the relationship between the amount of post-cue social contact with

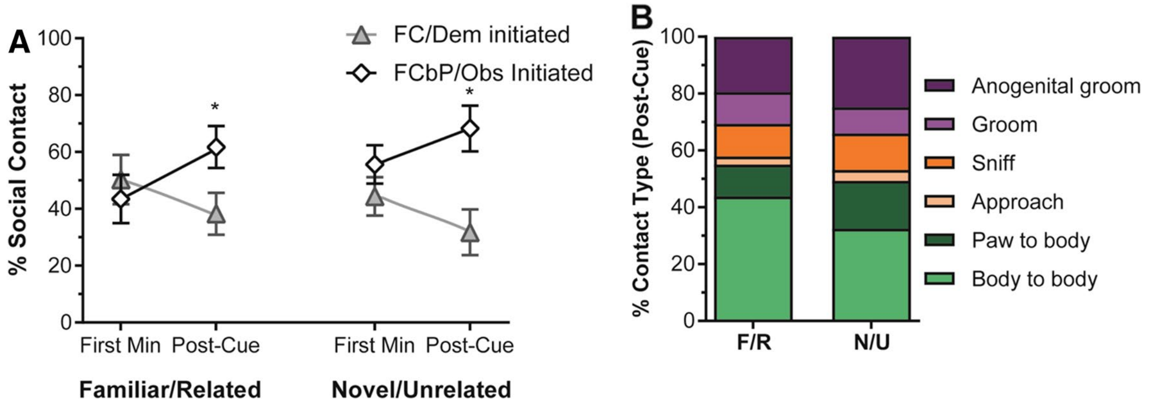

Fig. 7 Description of social contact on day 2. **a** Proportion of total social contact during first minute in chamber and 20 s post-cue initiated by FC/Dem rat or the FCbP/Obs rat. In both familiar/related pairs and novel/unrelated pairs, there was an increase in the proportion of social contact initiated by the observer after cue presentation. **b** Likelihood to engage in a specific type of social contact post-cue was similar between familiar/related pairs and novel/unrelated pairs

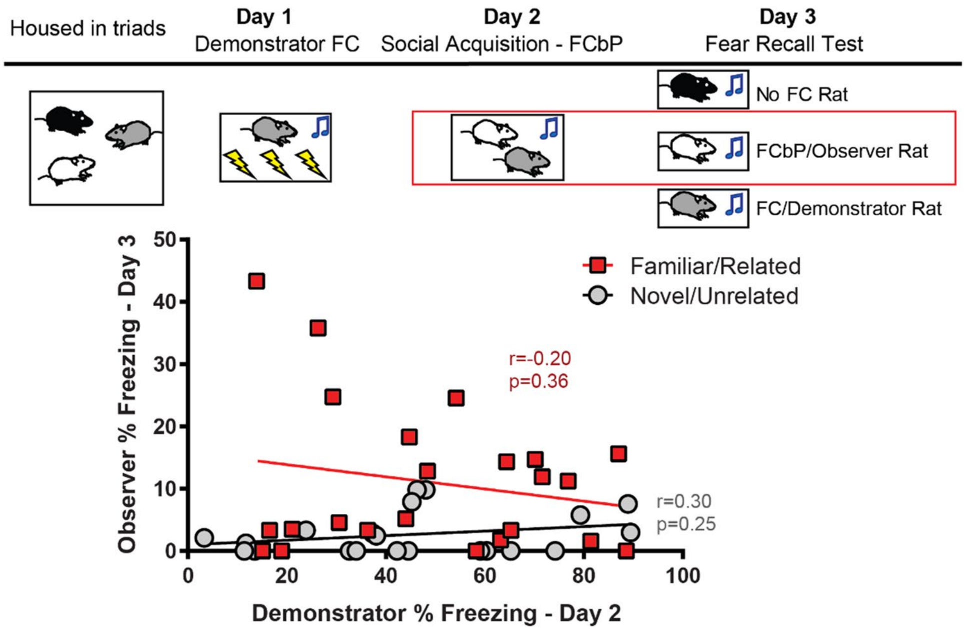

Fig. 8 FC/Dem rat day 2 freezing on day 3 FCbP/Obs rat freezing. Freezing displayed by the FC/Demonstrator rat did not predict freezing displayed by the FCbP/Observer rat during LTM tests the following day (both groups: ps > 0.2)

the demonstrator and expression of the learned behavior that was seen in FCbP observer rats with familiar sister demonstrators did not exist with novel demonstrators. These findings indicate that increased social contact alone is not sufficient to support social transmission of a fear association. In contrast, when we analyzed social contact during the interaction/acquisition period of the STFP social learning paradigm, there was no significant relationship between social contact directed towards the demonstrator and the observer's food preference. This was true for both STFP observers with familiar sister demonstrators and STFP observers with novel and unrelated demonstrators. In fact, in line with the findings of Galef and Whiskin (2008), observers with novel demonstrators showed a nonsignificant increase in preference for the demonstrated diet and a significantly greater amount of social contact towards their demonstrator. Taken together, these results may indicate that increased social learning with familiar demonstrators is unique to fear-based learning.

There are a number of potential explanations for the inconsistent effect of demonstrator familiarity between the STFP and FCbP social learning paradigms. One possibility is that observers learning via the STFP paradigm do not attend to demonstrator characteristics in the same way that rats in the FCbP social learning paradigm do. This interpretation is supported by a large amount of behavioral research examining the effect of various demonstrator characteristics potentially relevant to social learning. Such relevant characteristics as demonstrator age (Galef et al. 1984; Galef and Whiskin 2004), reliability (Agee and Monfils 2018), and health (Galef et al. 1983, 1990; Galef and Whiskin 2000; Grover et al. 1988) all do not affect the strength of the observer's preference for the demonstrated food. While similar research in social fear transmission is limited, there is evidence that demonstrator dominance also enhances learning of a fear association of Jones and Monfils (2016; Kavaliers et al. 2005), further indicating that demonstrator characteristics do partially mediate learning via FCbP. We and others have previously shown that there is increased social transmission of fear information when the demonstrator is socially dominant Jones and Monfils (2016; Kavaliers et al. 2005). In rats, dominant–subordinate relationships that result in increased social fear transmission in FCbP are characterized by increased social contact Jones and Monfils (2016). While social dominance was not measured in this current experiment, social contact initiated by the demonstrator towards the observer in the post-cue period could be a potential indicator of the dominant/subordinate relationship in rats and requires further study. Notable exceptions in STFP are demonstrator characteristics which promote increased investigation of the demonstrator or increased exposure to the carbon disulfide and food scent combination that mediates transmission. For example, as mentioned earlier, Galef and Whiskin (2008) found that observers learned better from novel demonstrators that were in competition with familiar demonstrators, an effect which was supported by a greater duration of the interaction between the observer and the novel demonstrator. It should be noted that while we did not observe a significant increase in consumption of the demonstrated food for rats in the Obs-NU condition over rats in the Obs-FR condition, our data did trend in that direction. Furthermore, Galef and Whiskin (2008) were only able to demonstrate this effect using a design in which observers had simultaneous access to both a familiar/related and novel/unrelated demonstrator that were isolated on separate sides of the interaction chamber. This design is naturally more sensitive to the observer's increased interest in a novel conspecific over a familiar conspecific.

A second possibility is that the enhanced acquisition of freezing behavior by familiar/related observers housed together in the FCbP paradigm could result from shared seemingly routine stressful colony situations (e.g., weaning, handling, cage changes, etc.), and may acquire a passive association between the fear- or stress-induced behavior of their cage-mate and the occurrence of these aversive events. As such, the effect of familiarity that is observed in FCbP may be the result of the second-order conditioning to the auditory cue driven by pre-formed associations between the demonstrator's fearful behavior and more mundane fear-inducing events. If this is the case, the effect of post-cue social contact on fear acquisition which we see in FCbP observers with familiar/related demonstrators may be indicative of an increased sensitivity to their demonstrator's fear behavior as a result of these associations. Furthermore, this would explain the lack of effect of familiarity/relatedness in STFP, as methodological differences between FCbP and STFP would make the second-order conditioning significantly less likely to occur in the latter paradigm. Specifically, in FCbP, the neutral auditory cue precedes a clear change in the demonstrator's behavior, facilitating associative learning between the cue and the demonstrator's distress. This is not the case for STFP in which the scent of food on a demonstrator's breath is present throughout the entirety of the interaction.

Similarly, it is possible that the effect of familiarity/kinship in FCbP is specifically mediated by an improved ability to recognize signs of distress in a familiar/related conspecific. While we did see similar amounts of post-cue interactions between observers regardless of whether their demonstrator was familiar or novel, the amount of post-cue interaction was only positively correlated with freezing on day 3 in observers with familiar/related demonstrators. As such, while increased post-cue contact might serve as an indicator of increased attention towards signs of distress in a demonstrator, social contact alone is not sufficient to recognize distress in a conspecific if that animal is not familiar. Given that we found group differences in social contact at the beginning of the FCbP session, it is difficult to parse out how these interactions may have altered later perception of the CS. As discussed earlier, in the STFP paradigm, most behavioral data points towards the conclusion that increased contact with and/or exposure to the breath of the demonstrator results in improved acquisition of the STFP. In line with this idea, Saggerson and Honey (2006) found that rats that had observed a trained conspecific pull a hanging chain at the presentation of a neutral cue to obtain a food reward were more likely to exhibit demonstrator consistent behavior when their demonstrator was not only socially novel but also from a visually distinct strain of rat. As such, it is likely that the results of this study are simply due to the different-strain demonstrators receiving increased attention from the observer during the acquisition phase as a result of their novel appearance.

Finally, the possibility that the increased freezing which we documented in observers in the FCbP paradigm may have arisen as a result of pre-exposure to the conditioning context and CS with a conspecific should be addressed. As the control rats in our paradigm received no such pre-exposure, with or without a conspecific present, this remains a potential explanation for our results. However, past research into the effects of social pre-exposure to context prior to fear conditioning in mice has found that pre-exposure with non-fearful demonstrators resulted in a buffering effect on subsequent fear conditioning that was not limited to observers with familiar demonstrators (Guzmán et al. 2009). While this past research would suggest that this is not the case, future work into the effect of pre-exposure specifically to conditioned stimuli with a non-fear-conditioned vs fear-conditioned conspecifics is still needed.

Conclusions

In summary, our findings provide further evidence that the effect of demonstrator familiarity/kinship is highly dependent on the social learning paradigm under examination. Our results also indicate that this dependence is likely supported by differences in the role of social contact between the social transmission of a fear and the social transmission of food preferences. The possible explanations put forth in our discussion are not mutually exclusive and may all contribute to explaining the observations from these experiments. Future research aimed at teasing apart the underlying neural mechanisms supporting the social transmission of both fear and food information is still necessary to fully understand exactly how demonstrator familiarity influences social learning.

Author contributions LAA designed experiment 1, gathered and analyzed data for experiment 1, and drafted the manuscript; CEJ designed experiment 2, gathered and analyzed data for experiment 2, and contributed to writing the manuscript; MHM designed the experiments, interpreted findings, contributed to writing, and approved the final version of this manuscript

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Data accessibility Our data and statistical analyses are readily available at: the University of Texas at Austin data repository, and may be found at the following link: https://dataverse.tdl.org/dataset.xhtml?persistentId=doi:10.18738/T8/0W9TZ9&version=DRAFT.

Compliance with ethical standards

Conflict of interest We have no competing interests.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards in accordance with the National Institute of Health's Guide for the Care and Use of Experimental Animals and were approved by the University of Texas at Austin Animal Care and Use Committee (#2018-00155).

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