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THE SOCIAL RELEASE PARADIGM: INVESTIGATIONS OF RESTRAINER AVERSION

PARADIGMA DE LIBERACIÓN SOCIAL: INVESTIGACIONES DE AVERSIÓN AL CONTENEDOR

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Abstract

In the social release paradigm, one rat is restrained in a plastic tube and a cagemate can open the restrainer door to release the rat from outside. Researchers have debated whether release is empathetically motivated or better understood as operant behavior reinforced by social contact. One fundamental assumption underlying interpretations of door opening in terms of helping or empathy is that being restrained in the tube is aversive to the trapped rat. The current study aimed to shed light on this controversy by investigating restrainer entries and re-entries by the free and formerly restrained rats after release, respectively. We hypothesized that if being restrained is aversive, the formerly

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restrained rat would make fewer restrainer re-entries following release than the free rat due to its aversive learning history with the restrainer. The results show that the free and formerly restrained rats explored the restrainer equally often following release, thus supporting prior research suggesting that being trapped in the restrainer is not aversive. The observation that the two rats made more positive 50kHz vocalizations before than after release also lend some support to this suggestion.

Keywords: Aversion, social release, pro-social behavior, 50kHz calls

Resumen

En el paradigma de la liberación social, una rata está sujeta en un tubo de plástico y un compañero de jaula puede abrir la puerta del contenedor para liberar a la rata desde el exterior. Los investigadores han debatido si la liberación está motivada por la empatía o se entiende mejor como una conducta operante reforzada por el contacto social. Una suposición fundamental que subyace a las interpretaciones de la apertura de la puerta en términos de ayuda o empatía es que la sujeción en el tubo es aversiva para la rata atrapada. El presente estudio pretende arrojar luz sobre esta controversia investigando las entradas y reentradas en el tubo de restricción por parte de las ratas libres y las ratas anteriormente sujetas tras su liberación, respectivamente. La hipótesis era que, si la sujeción resultaba aversiva, la rata que había estado sujeta haría menos reentradas en la jaula tras la liberación que la rata libre, debido a su historia de aprendizaje aversivo con la jaula. Los resultados muestran que las ratas libres y las que habían estado sujetas exploraron el contenedor con la misma frecuencia tras la liberación, lo que respalda investigaciones anteriores que sugieren que estar atrapado en el contenedor no es aversivo. La observación de que las dos ratas emitieron más vocalizaciones positivas de 50 kHz antes que después de la liberación también respalda esta sugerencia.

Palabras clave: Aversión, liberación social, conducta pro-social, vocalizaciones de 50kHz

Rodents are frequently used to model behavior and medical conditions across research fields in psychology, medicine, and biology. One animal model is based on social-release where one rat is restrained in a cylindrical tube and a cagemate can release it. The original paper (Bartal, et al., 2011), later echoed by others, claimed that this experimental paradigm tested rodent empathy (Bartal et al., 2014, 2016; Sato et al., 2015; Tomek et al., 2019). However, others have argued that social release is better described and explained as operant behavior controlled by social reinforcement (Hachiga et al., 2018; Hiura et al., 2018; Schwartz et al., 2017; Silberberg et al., 2013).

Fundamental to this debate is the question of whether being restrained is aversive. The interpretation of cagemate release in the social release paradigm as emphatic or pro-social rests on the assumption that restraining is stressful and aversive to the restrained rat, and that release relieves distress. Simply put, if the restrainer is aversive to the restrained rat, then releasing it can be interpreted as empathic; if it is not aversive to be restrained, then the empathic distress-based account for freeing the restrained rat loses some of its strength. The assumption of restraint stress is based on previous studies where a rat undergoes forced immobilization for a varied amount of time (Paré & Glavin, 1986). Restraint, or to keep the animal still while samples are collected, is often a part of procedures in studies where blood samples are taken (Paré & Glavin, 1986).

Numerous studies show that being restrained is associated with heightened levels of corticosteroid stress-hormones (Kalil et al., 2013) and increased levels of stress (reviewed in Buynitsky & Mostofsky, 2009). Restraining a rat can also affect feeding/foraging and exploration following release (Ely et al., 1997; Tu et al., 2019), and lead to avoidance of the location containing stressful cues (Nemati et al., 2013). Such avoidance of the location containing stressful cues, termed conditioned place aversion, has been extensively studied by giving an aversive stimulus (e.g., shock) when the animal is in a specific location in an arena/compartment. In this procedure, a stimulus' aversiveness is defined and assessed by its behavioral affects (i.e., reducing or eliminating behavior). Conditioned place aversion studies show that rats on later occasions will avoid areas where they have experienced a painful stimulus such as radiation (Garcia et al., 1957) or noxious gas (Ramsay et al., 2003),

In the social release paradigm, restrainer aversiveness can be operationalized and assessed by comparing the number of restrainer entries or the time spent in the restrainer in rats following restrainer release to the same measures observed in rats without a history of being restrained. Based on this operationalization, Silberberg et al. (2013) have challenged the claim of restraint aversiveness and the interpretation in Bartal et al.'s study that release was empathetically motivated (Bartal, Decety, et al., 2011). Silberberg et al. argue that being locked in the restrainer is not aversive because, following release, the rat would return to the restrainer despite its presumed aversiveness, and that release was driven by the pursuit of social contact (Silberberg et al., 2014). Hachiga et al (2020). went a step further and claimed that the restrainer is not only non-aversive for rats, but that being in the restrainer is reinforcing. Hachiga et al.'s. claim of restrainer non-aversiveness was based on latency and number of re-entries by the restrained rat after being released. Instead of experiencing being restrained one more time, the rats could explore a familiar tube-like structure, and it is this exploration the authors claim is reinforcing.

In the social release paradigm, the free rat usually has no experience with being restrained and the potential aversive aspects of it. Thus, restraint stress, fundamental to the empathic distress-based interpretation, needs to be conveyed from the restrained to the free rat through stimuli like pheromones, distress calls, postures, and movements. Conversely, without any external distress-signaling stimuli, one needs to assume that the free rat can attribute mental states and emotions to other individuals (theory of mind, ToM), which seems questionable. Consistent with recent research indicating multiple possible motivators for restrainer door opening by the free rat (Silva et al., 2020), variables such as odors, sounds, visual stimuli, in addition to procedural differences like housing conditions (e.g. in twos or triplets), response requirements, and differences in experimental arena may influence cagemate release (Blystad, 2019). Sound is perhaps of particular interest considering that rats are known to communicate affective states via ultrasonic vocalizations (USV). Positive states are conveyed with sounds around 50khz, and negative states around 20khz (Brudzynski, 2013). These two categories may serve different functions and elicit different kinds of behavior in the receiver; the 50khz is associated with social contact, and the 20khz is used to warn conspecifics of potential dangers, communicate social defeat (Seffer et al., 2014), and communicate fear (Kim et al., 2010). Additionally, within these two main categories, several others exist, possibly giving an opportunity for complex ultrasonic communication. Bartal and colleagues measured the 20khz USVs, and concluded that these were not relevant for restrainer opening (Bartal, Decety, et al., 2011). This finding conflicts with a recent study showing that ultrasonic vocalizations increased when the rats were not released. However, this study employed a somewhat different design using an escape platform and targeted helping instead of a restrainer opening up to an arena (Cox et al., 2022). The potential role of the 50kHz USV sounds in the social release paradigm has to our knowledge not been investigated but awaits future studies.

The current study represents a supplement and extension of the disagreement on restrainer aversiveness in the social release paradigm. The two main explanations for the release of the trapped rat can be evaluated by experimentally testing their underlying assumptions. The explanation of empathetic concern rests on the assumption of both restrainer aversion and the elicitation of a similar emotional state (emotional contagion) in the free rat. Assuming that the free rat is unable to attribute mental states and emotions to the restrained rat (ToM), emotional contagion is contingent on stimuli such as vocalizations, odor, or visual stimuli conveying restraint stress to the free rat. Data inconsistent with either restrainer aversion or emotional contagion are also inconsistent with the explanation of empathetic concern. The operant explanation, which suggests that release is positively or negatively reinforced by its consequences (social contact or termination of an aversive stimulus), requires neither restrainer aversion nor emotional contagion for the positive reinforcement explanation, and only restrainer aversion (and an aversive stimulus emitted by the trapped rat that is removed by release) for the negative reinforcement explanation.

The current study tested assumptions underlying the empathetic concern explanation for social release. As indicators of restrainer aversion or emotional contagion, the current study examined partial and full restrainer entries by the free and formerly restrained rats following release, and vocalizations made by the rats during the task. Measuring partial entries is an extension of Hachiga et al. (2020) study, and was prompted by the rationale that the restrainer door cannot be closed and the rat can escape much faster during partial restrainer compared to full restrainer entries. Thus, it is a possibility that restrainer aversiveness can differentially affect partial and full restrainer entries. Whether there is a difference between these two forms of restrainer entries is, of course, and empirical question. However, separating between and measuring these two forms offers a novel level of detail that could reveal difference in behavior between the free and previously restrained rat. The main hypothesis tested in our study was that, if being restrained is aversive, the restrained rat would enter the restrainer less frequently than the free rat following release. We also hypothesized that, if being restrained is aversive and restraint stress produces emotional contagion in the free rat, we will observe fewer positive vocalizations before release than after release.

Data were collected during a study on the effects of response shaping and restrainer content on opening latency using the helping behavior paradigm. This study has already been published and was not specifically designed to answer the question of restrainer aversiveness (Blystad et al., 2019). Early findings regarding the role of positive ultrasonic vocalizations (USV) are also included. However, methodological shortcomings (i.e., inability to identify the vocalizer, no differentiation between different kinds of positive USV, no recordings of negative USVs) do not allow for any conclusions regarding their possible influence.

Methods

Subjects and housing

Thirty female Sprague-Dawley rats, 100 days old and weighing between 150 and 200g, were purchased from Janvier, France. The animals were randomly divided into 15 pairs and housed in transparent cages (412 x 25 x 25). Cohabitation for 14 days began upon arrival at the animal facility in order to establish cagemate relations between the randomly coupled rats. After the cohabitation period in a single home cage, the rats were housed in separate cages, and one rat from each couple was food-deprived during behavioral training. Food deprivation lasted for a total of 10 days, and daily weighing ensured that no rat lost more than 15% of its free-feeding weight. Rats that were food-deprived were given smaller rations of standard chow and housed in adjacent cages to their previous cagemate to maintain social bonds during food deprivation and the separated living phase. This housing situation avoided depriving both animals of food, but enabled the animals to maintain social vocalization, transmission of odors, and observation of behavior. Additionally, the rats were given 1 hour per day to socialize in a neutral cage except during the weekend. Following food deprivation, the animals were housed together and given food and water ad lib. One couple was removed from the study due to deviant behavior caused by incorrect deprivation during the shaping procedure. Thus, 14 rat couples were included in the study.

The study was approved by the Norwegian Animal Research Committee (ID# 7966). All procedures for housing and euthanasia were performed at the Department of Biosciences at the University of Oslo (https://www.mn.uio.no/ibv/english/). Daily inspections by the main author, in addition to the in-house animal technicians and veterinarian ensured the animal welfare. All animals were euthanized with carbon dioxide gas. Video recordings from these rats have previously been analyzed and published (Blystad et al., 2019), however new analyses, and this publication, were justified by new research questions (Fine & Kurdek, 1994) namely effects of restrainer aversion and positive USVs. Reusing the video recordings also allows for reducing the number of animals needed, in line with the 3R ethical guidelines (NC3Rs, 2019).

Apparatus

A plastic box measuring 0.5m x 0.5m was used for this experiment. Matte black duct tape was used to cover the walls of the box. This was done to prevent mirror-like reflections. A thin metal pipe ran across the leftmost wall of the box. At the end of the pipe was a small square metal recipient that the food reinforcers (i.e., spherical 5mm food pellets) were administered in. Within the box a cylindrical plastic restrainer containing two doors on each side was used. Only one of the doors could be opened by either pressing a lever, tipping the door open with the paws or head, or tipping over the counterweight. Illustrations of the apparatus can be found in Blystad et al., (2019).

Eight students assisted in the experiments, and each of the rat-couples was assigned one set of handlers for the entirety of the experiment: two student laboratory assistants and the primary author. To minimize noise in the data, a detailed experimental protocol was developed, and all students underwent a training program in animal handling and experimental testing under the auspices of the main author. The laboratory assistants were continuously supervised by the main author to ensure that protocols for laboratory conduct, and experimental procedures were followed. The following measures were taken to reduce effects of single housing and food deprivation when proceeding to subsequent conditions: 1) The rats were allowed to play and socialize for 1 h each day in a separate cage and were housed in adjacent cages to maintain social bonds, and 2) 60 h of co-habitation and food ad lib took place before proceeding to the next condition.

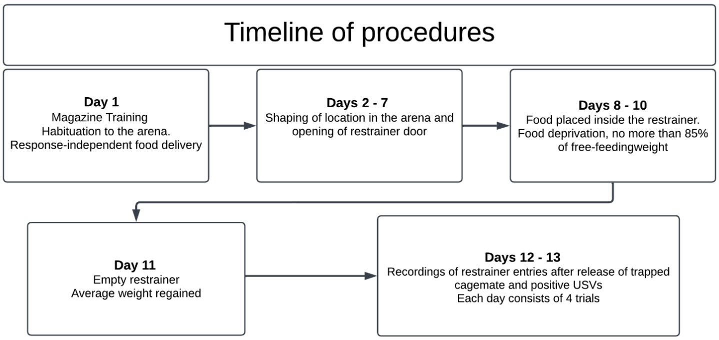
Procedures

The rats were randomly divided into two groups; free or restrained. One free and one restrained rat were then housed as cagemates apart from the period of food deprivation described above. All free rats in the current study went through habituation to the arena and testing in the same manner. This consisted of seven days of training and habituation before food was inserted into the restrainer. During this seven-day period, all rats opened the restrainer, thus demonstrating that the opening response was in their behavioral repertoire. For the period of training and habituation up until testing with a cagemate in the restrainer, the restrained rat remained in the homecage. Then, the rats were given 60 h of co-habitation and food ad lib, and a single day of testing with an empty restrainer before conditions with a restrained cagemate began. During these data collection trials, the restrained rat was first inserted into the arena. Immediately after, the free rat was inserted into the arena and the trial began. Thus, the minimum restrained time was the time it took to insert the free rat plus the door opening latency (approximately 10 seconds + door opening latency); the maximum restrained time was 5 minutes. The intertrial interval was 5 minutes, during which the rats would be placed in their homecage. These cagemate conditions were run across two consecutive days, where each day included four trials. The first of these four trials was excluded from the analyses to remove possible effects on behavior of being transported to the experimental room, and to allow the rats to familiarize themselves with the arena before testing.

Three rat couples only had one day of cagemate experiments as these were tested an additional day with an empty restrainer due to exceptionally short latencies during their first day with an empty restrainer. An analysis of data from these rats did not show any difference in terms of latency to open the restrainer and were thus included in the further analyses (see Blystad et al. 2019 for further details). Thus, all 14 rat couples completed the four trials during Day 1, but only 11 rat couples completed the four trials during Day 2. Data from the three couples which did not complete Day 2 were still included in the statistical analyses.

The procedure included more conditions than described above (see Blystad et al., 2019 for a more detailed description), and only the conditions relevant for the current article are described here. See timeline in Figure 1 below for a summarized overview of the procedures in the current paper (Figure1).

Figure 1. Flowchart overview of the procedures ending with the experimental days 12 and 13



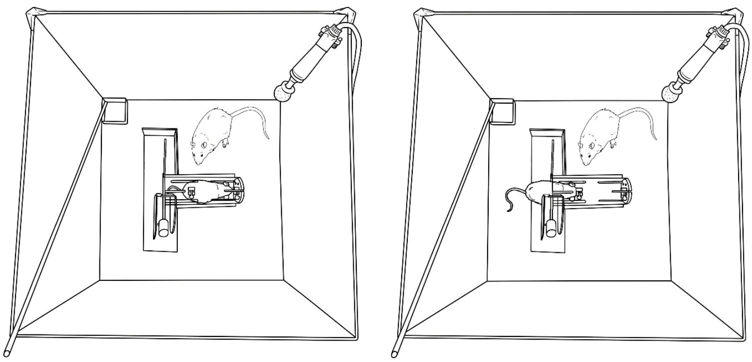
Note. The first trial of each session was excluded to avoid effects of the transportation to the experimentation room. Only trials with opening were analyzed. Three rat couples only completed Day 12, reducing the number of analyzed trials by 3 for each couple. Summarized 54 trials were analyzed for entries and USVs during Days 12-13.

Measures

Restrainer entries following release

Following door opening and release of the restrained rat, all rat couples were given 5 minutes to socialize. Video recordings of these 5 minutes were viewed by two independent observers who manually counted restrainer entries. Entries were divided into two categories: A full restrainer entry was defined as an entry with the entire body except the tail. All remaining entries where the rat had part of the body inside the restrainer were counted as partial entries (see Figure 2). The interobserver agreement was 98.6% for partial and 95.7% for full entries. The restrained rat was marked with a black line on the tail enabling the observers to separate the two rats during video-scoring. Prior to the experiments with a cagemate, the free rat underwent several phases of training and habituation ending with the demonstration of opening the restrainer to access the food inside and a session with an empty restrainer. For more details, see protocols for training and habituation and depiction of restrainer opening mechanism (Blystad et al., 2019).

Figure 2. The arena with the free and previously restrained rats following release



Note. The figure to the left illustrates a full entry of the restrainer, i.e., with the entire body. To the right is an example of a partial restrainer entry where only the front part of the body is inside the restrainer. Other items depicted in the figures include a pipe extending from a metal box in the arena for food reinforcement during training and habituation (left side of the arena) and an ultrasonic microphone for recording of USVs (top right corner). The figure is adapted from (Blystad et al., 2019).

USV recordings

Ultrasonic vocalizations were recorded with a bat detector (NHBS, 2015) that was connected to a high-frequency microphone mounted to one of the top corners of the experimental arena (see Figure 1). This bat detector collected all sounds in the frequency band upwards of 18Khz. These recordings were timestamped and measures before and after opening of the restrainer door.

The microphone was unable to identify which of the free and restrained rats made the vocalizations. Thus, all measures of vocalizations are from the pair of rats. A second methodological weakness is that between a pilot study and the start of the final experiment, a new and unforeseen source of sound pollution emerged in the laboratory or its surroundings. Unfortunately, this caused our recordings of negative USVs in the in 22 kHz band to be unanalyzable.

The recordings were analyzed in batch analyses of spectrograms with Avisoft SASLab Pro (Berlin, Germany) to collect the positive 50 kHz USVs. The cutoff frequency was set to 25 kHz, as undefined noise prevented data collection below this frequency. Furthermore, automatic whistle tracking was selected which collected USVs above 1ms in length. Number of USVs were copied to a Microsoft Office Excel spreadsheet. As the time before restrainer door opening differed between rats and sessions, rates of USVs (min^{-1}) were calculated to enable comparisons across conditions and animals. The analyses collected all positive USVs but did not differentiate between the different types of positive USV described in the literature (e.g., Burgdorf et al., 2011; Burke et al., 2021).

Statistical Analyses

Analyses of variance was done in Statistica (Statsoft Inc., v. 13.5.0.17) with the alpha level set to 0.05. Prior to statistical analyses, trials that did not include social release were discarded from the data set (30 out of 84 trials).

For all rats, the number of restrainer entries were averaged across trials. For 11 rat couples, entries were averaged across all six trials (Day 1 and 2). For the remaining three couples, entries were averaged across the three trials during Day 1. Restrainer entries were analyzed using factorial ANOVA with confinement status and entry-type (each with two levels – free or formerly restrained: partial or full restrainer entries) as the independent factors, and number of entries as the dependent variable. Tukey HSD post-hoc tests were used to evaluate significant interaction effects.

The ultrasonic vocalizations were analyzed by repeated measures ANOVA with the 12 conditions and before and after restrainer release as the repeated factors. The USV data did not permit for separating between the rats and thus is based on couples. The number of positive USVs per minute before and after restrainer release were compared using a repeated measures ANOVA with trials (6) and before/after (2) release as the within-subject factors. A single subject analysis was also made of a selected group of rat couples that had completed most of the trials to further illustrate the ANOVA results.

Lastly, the correlation analysis was done using Microsoft Excel (Microsoft 365) and investigated the association between the number of total positive USVs made prior to door opening during a trial and the time spent in the restrainer. This correlation analysis included all trials except those in which technical errors occurred during testing (16 trials total). Additionally, one extreme outlier was discarded, so the total number of trials included in the correlation analysis was 67.

Results

The main result is that the free and formerly restrained rats entered the restrainer equally often following release. At a more detailed level, the number of partial restrainer entries was statistically significantly higher than full restrainer entries in both groups. Also, the formerly restrained rats had significantly more partial restrainer entries than the free rats, but the number of full restrainer entries was not statistically significantly different between the groups (Figure 2). Lastly, the rats made more positive ultrasonic vocalizations before than after restrainer release.

Restrainer entries following release

The analysis of restrainer entries averaged across trials showed no effect of confinement status, $p = 0.64$, indicating that the free and formerly restrained rats entered the restrainer equally often following release (Figure3.) However, the analyses did show a statistically significant main effect of restrainer entry type (partial or full), $F(1,52) = 72.28$; $p < 0.0001$, $\eta^2 = 0.58$, CI [1.46, 2.37], with more partial than full entries. There was also a statistically significant confinement status x entry type interaction effect, $F(1,52) = 14.17$; $p = 0.001$. $\eta^2 = 0.21$, Post hoc Tukey HSD tests of this interaction effect showed that the number of partial entries in the formerly restrained rats was higher than in the free rats, $p < 0.05$, and also significantly higher than full entries in both free and restrained rats, $p < 0.001$. Number of partial entries in free rats was also statistically significantly higher than the number of full entries in both free and restrained rats, $p < 0.01$ and $p < 0.001$, respectively. Number of full restrainer entries was not statistically significantly different in free and formerly restrained rats, $p = 0.10$.

Figure 3. Partial and full restrainer entries

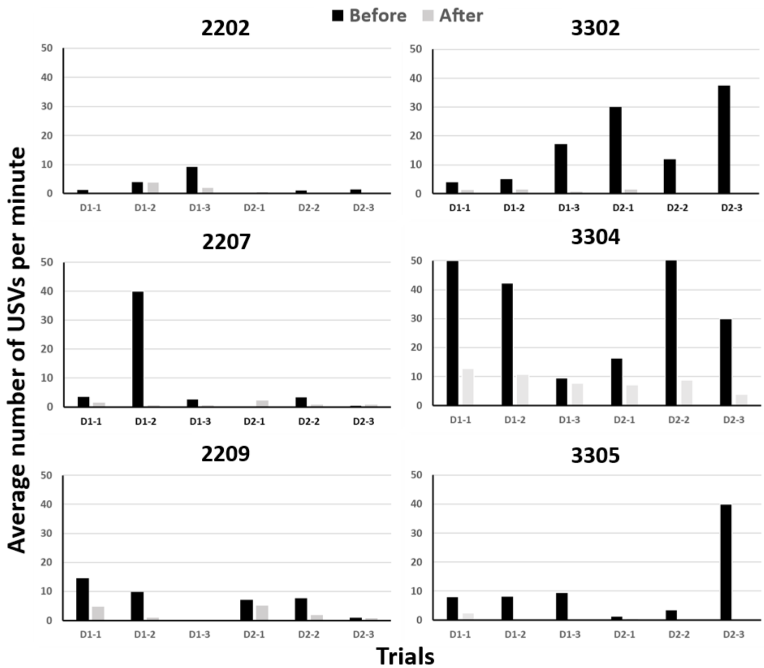
Note. Partial and full restrainer entries averaged across trials during both two days in restrained and free animals following release. The free and restrained rats both entered the restrainer after release, but the formerly restrained rats had more partial restrainer entries than the free rats, and both had more partial than full restrainer entries. Asterisks and lines denote significant differences, diamonds denote average scores.

Positive USVs before and after release: Average rates, examples of single subject analyses and correlations between number of USV and time before release from restrainer

The rate of positive USVs was statistically significantly higher before than after restrainer opening (17.4 min^{-1} and 2.8 min^{-1}, respectively), $F(1,13) = 7.85$; $p < 0.05$, $\eta^2 = 0.38$. The analyses also showed a statistically significant effect of trials, $F(5,65) = 2.78$; $p < 0.05$, $\eta^2 = 0.18$, and a statistically significant time x trials interaction effect, $F(5,65) = 2.91$; $p < 0.05$, $\eta^2 = 0.18$. These two statistically significant effects were carried by three extreme scores before restrainer opening on Day 1 (SD before and after opening were 20.2 min^{-1} and 2.3 min^{-1}, respectively). In follow-up analyses, the trials effect and time x trials interaction effect disappeared when the three extreme scores were substituted with average scores. However, substitution did not affect the analysis showing a statistically significantly higher rate of positive USVs before compared to after restrainer opening.

To further explore a possible function of USVs for door opening, we analyzed the correlation between the number of USVs before door opening and door opening latency. In this analysis, one outlier was removed. However, the inclusion of this data point did not alter the correlation analysis. The analysis showed no correlation ($r=0.04$) between USVs and latency ($R^2 = 0.0015$, $F(1,65) = 0.01$, $p = 0.78$). To illustrate the USV data, six rat couples were selected based on the criteria that restrainer door opening occurred on at least five of the six trials. Visual inspection of the data shows consistently the same effects for individual rat couples as seen at the group level, namely that more positive USVs were emitted before rather than after restrainer opening (Figure 4).

Figure 4. Single subject graphs of selected rat couples



Discussion

This study examined assumptions underlying the empathetic concern explanation for social release. Restrainer aversion and emotional contagion were assessed by observing and comparing number of restrainer entries by the free and formerly restrained rats following restrainer release, and positive ultrasonic vocalizations made by the rats before and after release. The results show that the free and formerly restrained rats had an equal total number of restrainer entries following release. More partial than full restrainer entries were observed in all rats, but this difference was more pronounced in the formerly restrained rats than in the free rats (Figure 2). Preliminary results from analyses of the positive USV recordings showed that the rats made more positive vocalizations before than after release (Figure 4), but there was no correlation between number of positive USVs prior to door opening and door opening latency. However, due to methodological weaknesses, these USV data are inconclusive and need replication.

The original study on social release did not include data showing number of restrainer entries after social release (Bartal, Decety, et al., 2011). However, a movie published as additional material to the study showed at least one instance of the free rat entering the restrainer after opening (Bartal, Mason, et al., 2011) raising the question of how aversive being restrained is in the social release paradigm. One study partially investigated this question and showed that the captive rat would re-enter the restrainer (Silberberg et al., 2013). However, the study lacked a direct comparison to number of entrances made by the free rat, and additionally used a design that did not allow for a direct comparison to the original study. Many studies have shown how restraining affects behavior and biology in rodents (Glavin et al., 1994; Paré & Glavin, 1986), but little is known about effects of restraining in the social release paradigm. Recently though, restrainer aversion was addressed by Hachiga et al. (2020), showing that restrainer did not seem aversive, but rather reinforcing.

The current data, showing a similar number of restrainer entries in the free and formerly restrained rats following release, seem to support the suggestion that being restrained is not aversive. However, several variables that may have affected restrainer entries following release were not controlled for in the present study design. In the following sections, we will explore different interpretations and explanations of our data, and how consistent they are with the suggestion of restrainer aversiveness and emotional contagion.

Learning history. In both the formerly restrained and free rats, previous learning history with the restrainer may have affected number of restrainer entries following release. The learning history of the formerly restrained rats is, of course, the issue at hand. A fundamental assumption in Hachiga et al.'s (2020) study and the current study is that re-entries following release from entrapment can be used as a measure of restrainer aversion. This seems a well-supported assumption given the literature on avoidance behavior (e.g., Nemati et al., 2013).

The free rat had a learning history with finding food inside the restrainer. Although this was followed by several extinction trials where the restrainer was empty, the free rat may have visited the restrainer following the release of a cagemate in later trials due to its learning history with food inside the restrainer. However, if this were the case, one would expect that the free rats had *more* restrainer visits following release than the formerly restrained rats. Thus, in the hypothetical case restrainer visits were not fully extinguished and the free rats visited the restrainer due to the previous learning history, a second variable not compatible with the claim of restrainer aversiveness is needed to explain why the number of restrainer entries was equally high in the formerly restrained rats without this food reinforcement history. It may be the case that being held in the restrainer was reinforcing as suggested by Hachiga (2020), which is antagonistic to the assumption of restrainer aversiveness.

Observational learning is, of course, related to learning history, and the similar number of entries in the free and restrained rats could be caused by observational learning (reviewed in Debiec & Olsson, 2017) whereby the free rats observe distress in the restrained rat and thereby respond to the restrainer in the same manner as the formerly restrained rat after release. This suggestion is consistent with restraint distress and emotional contagion. If correct, however, the similar number of restrainer entries implies that observational learning is equally effective as direct experience with being restrained. This is perhaps questionable.

General exploration. The rats may have entered the restrainer following release as part of general exploration of the experimental arena. The formerly restrained rats would be familiar with the restrainer and its odors, so fewer exploratory restrainer entries may be expected compared to free rats. If the current study had found fewer restrainer entries in the formerly restrained rats than the free rats, then exploration would be a possible confounding variable explaining the results as both general exploration and restrainer aversiveness predict more visits by the free rats. However, the number of restrainer visit was the same in the free and formerly restrained rats, thus, general exploration as an explanation of the results seems incompatible with the suggestion of restrainer aversiveness.

Partial or full restrainer entries. The higher proportion of partial restrainer entries in the formerly restrained than free rats may be interpreted as an indication of "caution" (i.e., positioning part of the body outside the restrainer to prevent being re-restrained) produced by experience being restrained. This suggestion needs further study but seem incompatible with our findings that neither the total number of restrainer visits nor the number of full restrainer visits were statistically significant different in the free and formerly restrained rats.

In summary, few of the reviewed variables can explain the current findings in a way consistent with restrainer aversiveness. Rather, contrary to the assumption of restrainer aversiveness, which is fundamental to interpretations of release in terms of empathy, our findings support the claim that being restrained is not aversive and that the restrainer may even be reinforcing as suggested by Hachiga et al. (2020). There are, however, methodological improvements that would increase the strength of this interpretation. It could be argued that the few and similar number of restrainer entries means that the restrainer is equally aversive to the free and formerly restrained rats regardless of prior experience. This is perhaps not likely, as similar tubes, but open in both ends, are routinely used to enrich the environment in rats' home cage. However, to test this suggestion and to have a comparison for restrainer visits, the test arena could contain another tube or enclosed area. It would also strengthen the study to include a more detailed analysis of the behavior taking place post release and check for independence in restrainer visits between the two rats. This behavior may have social functions where one rat will follow the other into the restrainer, leading to equal number of visits in both rats. The design may also be improved by requiring a minimum time spent in the restrainer before inserting the free rat. In the present study, the free rat was inserted into the arena immediately after the restrained rat was put into the restrainer. Waiting a longer, predetermined time period before inserting the free rat could affect the results and should be subjected to further studies.

The current study also recorded vocalizations during testing and found that the rats made significantly more positive vocalizations before than after restrainer release. A significant methodological weakness, however, is that we were unable to identify which of the free and the restrained rats made these calls. Still, at face value and irrespective of the caller, this finding seems inconsistent with the suggestions of restrainer aversion and emotional contagion. For the restrained rat, a higher rate of positive calls while being restrained than after being freed is inconsistent with restrainer aversion. For the free rat, more positive calls when the other rat is restrained than after its release seem inconsistent with emotional contagion of restraint stress (although these calls may have been made as a form of consolation). It is unclear what role the positive USVs play in the social release paradigm, as there was no correlation between number of positive USVs prior to door opening and door opening latency (and amount of time the trapped rat spent in the restrainer). However, due to the methodological weaknesses of not being able to identify the caller, not differentiating between different kinds of positive calls, and, most importantly, having no recordings of negative USVs, our sound data should be interpreted with extreme caution. Positive and negative vocalizations need to be examined in future studies with improved experimental procedures.

Conclusion

The study showed that the free and formerly restrained rats made the same number of restrainer entries following the trapped rat's release from the restrainer. This finding is inconsistent with restrainer aversion, which predicts that the formerly restrained rats would make fewer restrainer entries than the free rats. Thus, the suggestion that restrainer door opening in the social release paradigm is empathetically motivated is not supported by the current data. Future studies should systematically explore vocalizations, odors, postures, and movement that potentially convey restraint distress from the trapped to the free rat to increase understanding of controlling variables, and to exclude theory of mind explanations of social release.

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