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Social Buffering as a Tool for Improving Rodent Welfare

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The presence of a conspecific can be calming to some species of animal during stress, a phenomenon known as social buffering. For rodents, social buffering can reduce the perception of and reaction to aversive experiences. With a companion, animals may be less frightened in conditioned fear paradigms, experience faster wound healing, show reduced corticosterone responses to novelty, and become more resilient to everyday stressors like cage-cleaning. Social buffering works in diverse ways across species and life stages. For example, social buffering may rely on specific bonds and interactions between individuals, whereas in other cases, the mere presence of conspecific cues may reduce isolation stress. Social buffering has diverse practical applications for enhancing rodent wellbeing (some of which can be immediately applied, while others need further development via welfare-oriented research). Appropriate social housing will generally increase rodents' abilities to cope with challenges, with affiliative cage mates being the most effective buffers. Thus, when rodents are scheduled to experience distressing research procedures, ensuring that their home lives supply high degrees of affiliative, low stress social contact can be an effective refinement. Furthermore, social buffering research illustrates the stress of acute isolation: stressors experienced outside the cage may thus be less impactful if a companion is present. If a companion cannot be provided for subjects exposed to out-of-cage stressors, odors from unstressed animals can help ameliorate stress, as can proxies such as pieces of synthetic fur. Finally, in cases involving conditioned fear (the learned expectation of harm), newly providing social contact during exposure to negative conditioned stimuli (CS) can modify the CS such that for research rodents repeatedly exposed to aversive stimuli, adding conspecific contact can reduce their conditioned fear. Ultimately, these benefits of social buffering should inspire the use of creative techniques to reduce the impact of stressful procedures on laboratory rodents, so enhancing their welfare.

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Individuals exposed to acute aversive experiences often react less to negative stimuli if they have social companions, an effect called 'social buffering.' Social buffering in rodents has commonly been studied in research that has targeted either fundamental, basic science aims or the applied aims of better understanding human health. However, social buffering also has applied welfare implications for improving animals' care and refining the impact of procedures, because it represents a way in which animals exposed to harms can be rendered more able to cope with them. These welfare implications are not always recognized or capitalized on, yet for rodents (our focus here) social buffering can be used to improve husbandry and to refine research procedures.

To encourage this, the first section of the current manuscript discusses the definition of social buffering, and the second describes how social buffering can affect animal welfare. The third section describes how social buffering is studied by reviewing the 2 aspects of experimental design that typically vary: the use of conditioned (acquired or learned) versus unconditioned challenges, and the presence of companionship in the home cage before or after a stressor, versus companionship *during* exposure to acute stress. This section also discusses how the buffering of animals subjected to stress (the 'subjects') is affected by the nature of the conspecifics placed with them (the 'associates') or the cues from such animals. The fourth section discusses how these associates and factors such as their behavior, stress levels and relationship to the subject may, in turn, modify their influence on the subject's responses. The fifth section summarizes the main affective and cognitive processes at work. Finally, the sixth section translates these studies into practical recommendations for using social buffering to improve the welfare of rodents in research. Here, some suggestions can be applied immediately, while others generate new testable hypotheses regarding welfare.

What is social buffering and why is it important?

Initially, social buffering was primarily studied in humans, and was called "stress-buffering" before the term "social buffering" appeared.[20,88] The work in humans inspired much subsequent research on laboratory animals, often with the aim to investigate underlying mechanisms of the human phenomenon. This research led to some refinements of the original definition. First, social buffering now refers to how the presence of *affiliative* social partners mitigates stress responses, where 'affiliative' refers not only to bonded individuals, but also more broadly to all "classes of partners that have evolved to interact amiably with each other even if the particular individuals are unfamiliar to each other".[55] Second, social buffering is now recognized as being mediated via multiple processes, most critically by changing the subject's assessment of the challenge (potentially with subcortical effects, such as changes in hypothalamic-pituitary-adrenal axis [HPA] reactivity).[41] Consequently, not all superficially stress- or pain-reducing effects of social contact warrant the term 'buffering'. For example, in rodents, being defeated by an aggressor can induce analgesia.[75] However, to-

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day this is not considered to be true social buffering because the causal interactions were negative, and because other endocrine, autonomic or behavioral responses to challenge are not reduced (and indeed subject anxiety typically *increases*).[55,75]

True buffering has been observed in many different species, including rhesus monkeys, goats, dogs, chickens, fish, and various rodents:[17,25,27,69,91,98,99] Norway rats, *Rattus norvegicus*, have been investigated the most comprehensively. For example, rats feature in one of the oldest experiments demonstrating a social buffering-type effect[81], and in a large corpus of influential research by Kiyokawa and colleagues.[19,60,72,81] Social buffering in guinea pigs (*Cavia porcellus*, plus wild species *Cavia aperea* & *Galea monasteriensis*) has been well-studied too.[32,36,40,43] Siberian hamsters (*Phodopus sungorus*), prairie voles (*Microtus ochrogaster*), degus (*Octodon degus*) and mice (*Peromyscus californicus, Peromyscus eremicus*, *Peromyscus leucopus,* plus several *Mus musculus* laboratory strains (CD1, C57BL/6/Sca inbred, BALB/c, C3H, and ICR), have also been studied, although to a lesser extent and often without being termed social buffering.[21,22,29-31,53,80,87,100] Collectively, these studies often document substantial effects of social buffering. Compared with control animals exposed to challenges when alone, the presence of an associate reduced cortiosteroid concentrations by 50% or more in guinea pigs placed in a novel environment, in Siberian hamsters subjected to wounding, and in rats exposed to "conditioned fear".[21,46,56,59,77] Freezing behavior was even abolished entirely in the latter animals. Furthermore, in hamsters and mice who are experimentally wounded (for example, having a section of skin removed using a biopsy tool), healing is accelerated (pair-housed hamsters healed completely in 11 d as compared with 13 d for hamsters housed alone) and analgesic self-administration was halved, indicating less pain.[21,80]

This rodent research also reveals that social buffering effects can vary greatly in magnitude. For example, using different strengths of current when shocking subject rats can alter the effectiveness of social buffering: for subjects shocked at greater intensities, the presence of associates had rather little impact, suggesting that social buffering is not a panacea.[59] Social context may also have little buffering effect for rats experiencing negligible amounts of fear; thus floor effects may be important too.[56] Sex can also alter the effects. In male and female prairie voles exposed to 10 d of chronic variable stress, females benefitted more from companionship than did males.[71] Social buffering may not be evident at all in some situations. For example, mice experiencing pain displayed no difference in apparent negative affect (for example, writhing) if housed alone or with an associate.[67,87] Likewise, male deer mice (*P. californicus*) paired with various conspecifics and exposed to chronic variable stress for 7 d showed no social buffering.[22] In rare cases, the presence of an associate can even *exacerbate* stress.[2-4,66,87] For example, BALB/c and C3H male mice injected with painful solutions may exhibit *hyper*algesia (manifested as increased writhing or locomotion) if placed with same-strain associates, compared with subjects housed alone after injection – the opposite of a buffering effect.[66,87] Three studies demonstrate a similar phenomenon in rats:[2-4] male Sprague–Dawley had higher corticosterone levels when placed in a novel environment with associates as compared with being placed there alone.

This variation and complexity of social buffering reflects 2 main issues, as we review in the sections that follow. The first is that an affiliation between subject and associate is crucial. However, the nature of this affiliation can vary in type and degree, and buffering studies vary greatly in both the social structures of the species used and the nature of the relationship between subject and associate. The second is that social buffering is not a single unitary phenomenon but rather is mediated by heterogeneous processes. Thus, different forms of buffering seem to overlap with a variety of related concepts such as isolation stress, emotional contagion, and stress resilience.

How social buffering is studied: Principal types of experimental design

Social buffering is studied by diverse approaches that vary in the type of challenge the subject is exposed to, the stress response measured, the nature of the associate, and how subject and associate are presented to each other. Two key distinctions reviewed here are whether the stressful challenge involves conditioning, and whether subjects encounter the associates before, during, or after this challenge. For consistency, 'subjects' are the animals experiencing stressful challenges, while 'associates' are the companions placed with them. This terminology will be used throughout and was adopted from some of the leading social buffering literature.[55]

The use of conditioned compared with unconditioned aversive stimuli. Whether the challenge involves conditioning (i.e. associative learning) is a major methodological distinction in social buffering research. In several examples mentioned previously, subjects were exposed to primary negative reinforcers (unconditioned aversive stimuli such as novel environments or wounding). However, a common alternative is to use conditioned fear as the challenge. In this situation, subjects are repeatedly exposed to a cue (for example, a novel environment) that is paired with a negative reinforcer (such as a shock). This cue therefore becomes a conditioned stimulus (CS) that itself induces fear and stress. Buffering is then typically studied in terms of the magnitude of subjects' responses during fear tests that involve exposure to this CS alone (for example, their duration of freezing, corticosterone levels, or extent of amygdala activation[28,33,56,61,64,73,74,77]). In this type of challenge, social buffering can also be inferred from cognitive measures, particularly the speed with which subjects extinguish their fear response to the CS after repeated exposure without shock.[9,15,74]

Housing-type compared with exposure-type social buffering. A second major methodological distinction reflects the location and timing of exposure to associates. Two principal subtypes of buffering have been proposed: 'housing-type' and 'exposure-type'.[54] In exposure-type social buffering, which is used more commonly, the presence of an associate *during* exposure to the aversive stimulus (often experienced outside of the home cage) causes subjects to respond with less fear or stress.[5,33,54] In housing-type social buffering, subjects are pair- or group-housed with associates before and/or after a challenge, but are alone when exposed to the challenge. In this situation, socially-housed subjects typically show less reaction to the aversive stimulus (for example, faster recovery) than do animals housed individually.[54,61,65]

The distinction between exposure- and housing-type social buffering is important, but this is not a simple dichotomy. For one, housing-type buffering itself comprises 3 different forms, based on whether social housing occurs before the challenge, after the challenge, or both. Furthermore, some manipulations that do not represent either of these subtypes also have effects. For example, one research group used fear conditioning in which associates were present only *during the fear conditioning phase*,[15] thus turning a typical exposure-type conditioned-based study (in which associates are present only during fear testing) into a housing-type study in which associates were present before the measurement of fear.[15] Likewise, when researchers induce wounds and then study healing, elements of both housing- and exposure-type buffering are involved.[21,8,94] Associates are never present during the *act* of wounding,[19] making such experiments housing-type, yet they *are* present during the subsequent pain, making these studies also exposure-type. Finally, exposure- and housing-type buffering effects are not mutually exclusive; they can be manipulated independently, and even combined (with associates being provided as cage-mates *and* as companions during challenge). For example, one group exposed rat subjects to an auditory conditioned stimulus (CS) previously associated with shocks.[63] Rats who were alone responded by freezing and showing stress-induced hyperthermia. Pair-exposure to the CS eliminated the behavioral, but not the autonomic, response: pair-housing for 24 h after fear conditioning suppressed the autonomic, but not behavioral, response, and subjects who were pair-housed and *also* pair-exposed showed no responses at all, as if combining housing- and exposure-type buffering completely blocked their fear conditioning.[63]

How the nature of the associate animal affects social buffering

As reviewed in this next section, several attributes of the associates influence the degree to which they benefit stressed subjects, with 3 general principles emerging from the literature. The first is that "dose response" effects are seen, with effectiveness being increased by qualitatively or quantitatively greater degrees of exposure to associates. The second is that, at least in some species, strongly affiliative or bonded relationships are often more effective (or sometimes even *required* for effectiveness) as compared with more neutral associates. For example, in naturally monogamous species, pair-bonds seem essential for effective buffering. Finally, stressed or anxious affiliates are generally less effective at social buffering as compared to calm associates (and are sometimes even counter-productive).[9,19,34,60,62,64,66,75,76]

Details follow, with Figure 1 summarizing some of these findings for rats.

Dose-response effects: Effectiveness increases with fuller, longer contact with more associates. In non-rodents, highly social species experience more effective buffering with multiple associates than with fewer, and the same pattern emerges in some naturally colonial rodents.[41] For example, exposure-type buffering studies on mice find that having multiple associates is better for reducing fear and anxiety: subjects with[76] 2 associates showed less freezing in a novel environment, and more use of open arms in elevated plus mazes, than did subjects with only one associate.[15] In rats exposed to conditioned fear, placing 3 associates with the subject during the fear expression phase also reduced freezing behavior more effectively than if just one associate was present.[59] Likewise, in housing-type studies, rats housed 4 per cage showed smaller autonomic responses to acute procedures than did rats housed alone, with pair-housed rats having intermediate responses.[84-81]

For housing-type social buffering, the duration and timing of social housing can be influential. Rats housed alone for longer periods than they were socially housed (12 h of social housing, followed by 24 h of isolation) no longer experienced buffering when exposed to conditioned fear.[58] In contrast, 12 h of previous social housing without an intervening period of isolation was effective.[58] In another study, male rats were fear-conditioned and either housed alone for 24 h, group-housed for 24 h, or group-housed for just the first 6 h or the last 6, 12, or 18 h.[65] Fear expression tests were performed 24 h after the start of these housing regimens. Social housing for the first or last 6 h in the 24-h period after fear conditioning did not mitigate conditioned hyperthermia to the CS, but social housing for over 12 h was effective. Likewise, in an exposure-type paradigm using male ICR mice, the associate had to be present for the full hour, during a 1-h period of physical restraint, in order to reduce markers of stress-induced cognitive impairment (phosphorylated ERK1/2 in the prefrontal cortex).[53]

Whether subjects have full contact with normally behaving conspecifics is another important factor. A study examining housing-type social buffering in mice with artificial wounds found that subjects allowed to interact physically with associates

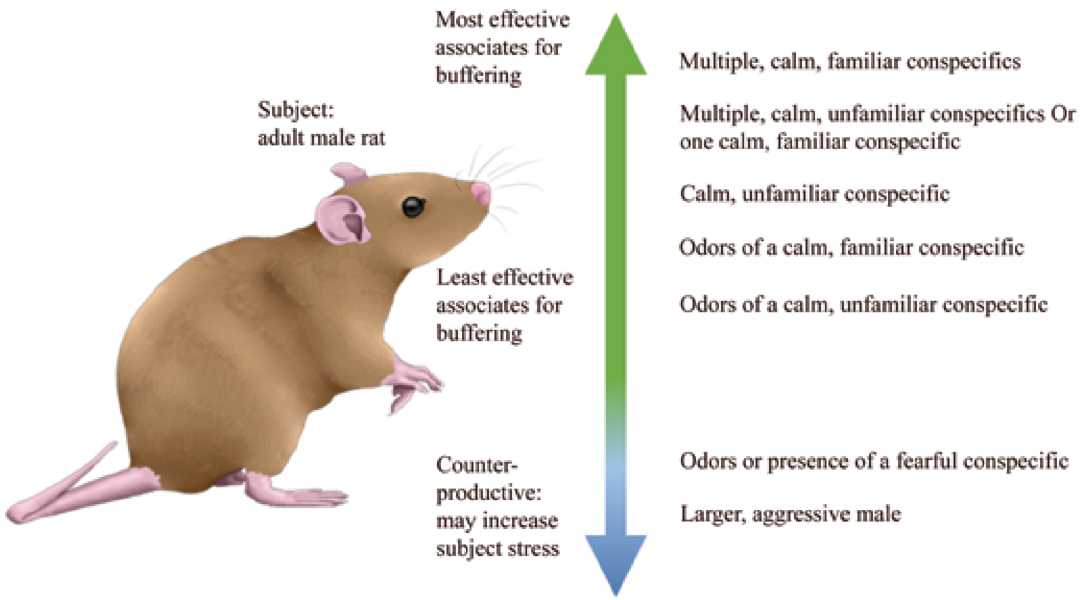


Figure 1. Many attributes of associate animals can influence their effectiveness as social buffers. The rankings shown here are for just a subset of all possible subject-associate combinations, but illustrate some key principles for rats.

recovered faster from surgery than did mice whose associates were presented behind bars.[94] Likewise, the prolactin response of juvenile rats to novelty stress varied negatively with the amount of play with associates, such that prolactin was least reduced when associates were behind bars and most reduced when subjects could interact physically with their associates[97] (although not all studies find the same).[13] In guinea pigs, female pups in a novel environment with their mothers had lower cortisol responses than did pups whose mothers were behind a wire mesh barrier (although the latter subjects still had lower cortisol levels than pups whose mothers were not present at all).[37] Unfamiliar adult male guinea pigs also reduced plasma cortisol elevations in young animals placed in a novel enclosure, but this reduction did not occur if the males were unconscious.[45]

Even the sight, sound or odor of associates may sometimes have buffering effects, at least to a degree. For example, stress responses of rats can be reduced by exposure to associates' odors alone (full body odors, potentially including feces and urine), although these are less effective than the actual presence of associates.[57] In other examples, however, subject mice placed in novel environments with just the bodily secretions of associates (urine, feces and plantar gland odors) had similar reductions in c-Fos positive cells in the paraventricular nucleus (PVN) of the hypothalamus (suggesting a reduced stress response) as did mice placed there with full associate contact. Physical exposure to associates, their urine, or a pheromone mimicking their urine likewise all caused similar reductions in subject mouse freezing behavior and extinction learning when exposed to a CS.[9,64] Auditory cues can also have buffering effects. In degu pups, several minutes of isolation in a novel environment for several days upregulated dopamine and serotonin receptors in the cortex, hippocampus, and amygdala: effects almost entirely prevented if pups were exposed to maternal calls during the challenge.[54,100-102]

Relationship effects: Familiarity and bonding increase an associate's ability to buffer. Social buffering may be improved by (or even dependent on) distressed subjects having a relationship with known associates.[38,55] The nature of an effective relationship will differ depending on the natural social structure of the species. Naturally promiscuous or polygynous group-living species form new relationships quickly. Other species instead form monogamous or close familial bonds that cannot be easily lost, gained, or substituted.[33] Thus, in adult guinea pigs, which form harems of multiple females and one male, females housed in triads of 2 females and one male were effectively buffered in a novel environment only by the bonded male, but not by an unfamiliar male.[41,46] Results were similar for males with bonded females: they were more effectively buffered than were males with nonbonded females.[41,46] In rats, unfamiliar associates can act as buffers, but their buffering influence is typically weaker than that of familiar associates.[57,90] Similarly, stressed female Siberian hamsters (either artificially wounded or stressed by physical restraint) had better healing rates and lower cortisol levels if they were placed with siblings rather than placed with unfamiliar associates, although the unfamiliar associates were still better than isolation.[21] Thus for guinea pig pups in a novel enclosure, the strong buffering effects of mothers occur even if mothers were unconscious,[44] while, as we saw above,[41,46] the buffering effects of unfamiliar adult male guinea pigs are weaker, in that they rely on these males being awake and active.[45] The strain of the associate can also influence effectiveness: odors from various rat strains differed in their effectiveness in buffering a subject's fear, with effective odors being derived only from strains similar to the subjects.[77]

However, some counter-examples have also been published. One lab found that adult rats showed *less* reactivity to a novel environment if with nonfamiliar conspecifics rather than familiar ones.[3,4] Familiarity can also influence the degree to which the subject influences the associate, via "emotional contagion" whereby one animal begins to mimic the affective state of another. For example, in mice, familiar animals may be more able to negatively influence each other. Thus, male mice injected with acetic acid would writhe in pain for a longer time if their associate (also injected with acetic acid) was their sibling than if the pair were strangers; and mice in pain display more pain behavior in the presence of painful cage mates than with painful strangers.[66,75]

The effects of associates can also be influenced by the natural social structure of a species.[41,55] Thus for the pups of guinea pigs and monogamous cavies, the mother is either the only, or the most effective, female associate able to act as a buffer:[32] infants have lower cortisol responses in a novel environment in the presence of their mother, compared to if their associate is an unfamiliar female.[40,41] In contrast, rats, which nurse communally, show no evidence of specific filial attachments: all lactating females seem to provide similar benefits.[41,55] Likewise, in monogamous species, buffering is often reliant on the associate being pair-bonded. Monogamous deer mice experienced buffering effects (evidenced by accelerated wound healing and lower corticosterone levels) when housed with an opposite-sex mate (their bonded partner), whereas polygynous species did *not* show buffering effects when housed with an opposite-sex conspecific (a non-bonded potential mate).[30,52] Furthermore, in monogamous prairie voles, only pair-bonded mates, but not other same-sex conspecifics, could produce buffering effects.[47] Such effects of the social system hold true across the subject animal's life; thus the unique value of the mother for buffering fear in young guinea pigs wanes as the subject ages, with non-mother female associates later becoming as effective as mothers at stress buffering.[32,39,42,46]

Affective state: Calm buffers are usually more effective (though perhaps not for male mice). Further highlighting the importance of emotional contagion, literature in rats indicates that non-fearful associates are generally better buffers than are fearful ones.[19,60,62] One research group showed the following gradation in how much freezing rat subjects display when exposed to a CS, from least to most: a fearful subject with a non-fearful associate, a fearful subject and associate, and finally a fearful subject alone.[19,60,62] Likewise, in male C57BL/6N mice, only subjects with non-fearful associates showed reduced freezing and faster extinction in conditioned fear tests; fearful associates had no buffering effects at all.[34,64]

Indeed, fearful or painful associates can sometimes be counter-productive: rather than merely being ineffective buffers, they can *increase* a subjects' stress. As one group summarized for rats and mice, "[pre-]exposure to a frightened demonstrator … shifted the effects towards fear promotion — a naïve conspecific had no effects, but a frightened one facilitated subsequent fear learning", attributing such a shift to "fear promoting cues emitted by a frightened demonstrator."[76] The same can hold for pain: "mice in pain, tested in the presence of cage mates also in pain, displayed more pain behavior than mice tested alone. …. Social communication of pain seemed to be mediated visually… which we concluded represented emotional contagion of pain from one mouse to the other."[75] Thus, male mice injected with a low dose of acetic acid, and presented with associate mice who had themselves received a higher dose, spent more time licking their injected areas.[66]

However, male mice can show contradictory results: the affective state of associates may have the opposite effects of those predicted by emotional contagion. One study used conditioned fear and a housing-type paradigm to study the speed with which subjects underwent extinction training to the CS (quantified by freezing).[9] Only fear-conditioned associates, and not those that experienced only the auditory tone, provided a buffering effect: thus fear-conditioned associates *reduced* the subjects' fearful behaviors to the CS and facilitated their extinction training.[9] These findings suggest that male mouse subjects may sometimes experience a better buffer effect if their associates are experiencing negative affective states rather than neutral ones.

The processes underlying social buffering effects

Here, we outline the diverse processes known or suspected to underlie social buffering. We do not cover the molecular mechanisms, which include central oxytocin and opioids and are reviewed thoroughly elsewhere.[52,92] Instead, we focus on higher-level (for example, cognitive) processes like appraisal, as these can more readily lead to practical recommendations for improving rodent welfare.

Acute isolation as a stressor. In exposure-type buffering, animals who are alone when presented with a challenge show greater responses than if associates are present: they react as if the challenge is larger in magnitude. This is not surprising: for many social species, simply being isolated is aversive, even without an additional challenge. Removal of subjects from specific bonded or attachment figures (for example, mothers or monogamous mates) can also trigger separation anxiety.[49,52,70] Furthermore, social rodents are often more motivated for social contact when they are stressed, likely reflecting how sociality in this taxon evolved as a "safety in numbers" strategy against predation.[7,23,24,52] Subjects exposed to challenges while also being isolated are thus being exposed to a compound stressor (for example, "pain + isolation", or "fear + isolation", as compared with just "pain" or "fear").

Decreased resilience: A lasting effect of inadequate social contact. In some forms of housing-type buffering, subjects previously housed alone show more reactivity to challenge than do subjects previously housed socially, despite no differences in how they are exposed to the challenge. Thus, social housing has a lasting effect that increases animals' abilities to cope with stressors, or isolation housing has a lasting effect that decreases such abilities. The latter interpretation reflects considerable evidence that chronic isolation is a stressor for many rodents.[7] Like the adverse effects of acute isolation outlined above, this interpretation arises in part from the powerful evolutionary benefits of social living for the ancestors of most laboratory rodents. For such species, isolation is unnatural and a threat to survival. In addition, small-bodied animals like mice living in highly ventilated IVCs, or in rooms whose ambient temperatures are set for human comfort rather than rodent thermoneutral zones, benefit in terms of thermoregulation from living with conspecifics, because this supports social huddling.[18,89] Overall, pre-stressor housing thus affects resilience, defined as "the adaptive maintenance of normal physiology, development and behavior in the face of pronounced stress and adversity".[7,14,79]

These beneficial carry-over effects of social housing join a variety of rearing- and housing-related factors that enhance rodent resilience, including receiving high levels of maternal care in infancy and living in housing conditions 'enriched' with physical complexity, preferred sensory stimuli and opportunities for exercise.[7,73,83,96] Such effects (typically evident even before challenge exposure as changes in baseline HPA activity), often seem to involve changes in threat appraisal, potentially rendering acute stressors less threatening and more controllable.[41,52] They also commonly manifest as altered levels of anxiety, with isolation-housed rodents showing more anxiety than socially-housed peers.[7,68] This in turn may reflect broader changes in judgment bias or cognitive bias; indeed rodents from barren housing do often interpret ambiguous cues less "optimistically" and more "pessimistically" than do rodents from physically enriched housing.[8,82]

Active effects of associates: Providing distraction, comfort and positive emotional contagion. The processes above represent relatively passive roles of associates, and are 'mere presence' effects that diminish stress from acute or chronic isolation. However, for some species or some stages of development, associates can play *active* roles in stress reduction via distraction, safety signals, and comforting interactions. This helps explain why different types of associates vary in their efficacy as buffers, and may be important in both exposure-type studies and housing-type studies in which social contact occurs *after* a challenge.

Associates may thus sometimes act as buffers simply by providing distraction, redirecting attention away from the challenge. Indeed, this explanation was offered in the very first research on social buffering, which described social contact as triggering rats' "exploratory drives" and thus distracting them from fear.[55,76] Furthermore, when guinea pigs pups are placed in a novel environment, their stress reactions are reduced if an adult is with them,[55] but the nature of this effect depends on the type of adult: as mentioned previously, if the adult is their mother, she is effective even if unconscious, but a male has a buffering effect only if conscious. Male interactions with pups are notably "vigorous", and the pups' reduced stress reactions in this situation may reflect the male providing a distraction (rather than comfort).[45] Finally, in a review of 3 few unusual rat studies that found more effective buffering by unfamiliar rather than familiar conspecifics, one group stated that "this is thought to be due to a higher novelty effect of the environment (i.e., new location) when the animal is placed there with a familiar conspecific that it can ignore."[49]

Associates may also act as buffers by communicating positive emotions. Above we discussed how emotional contagion can reduce buffer effectiveness if associates are stressed. However, emotional contagion can also be positive because rodents can generate positive social cues that provide safety signals (for example, via odors from the neck in rats).[76] Rodents may also comfort each other actively through physical interaction, although studies of this for adult animals (rather than the licking and grooming of infants by mothers) are rare. For example, in the monogamous prairie vole, buffering effects may depend on associates directing "consolation" actions, like allogrooming, to subjects.[10,55] Likewise, one author made this intriguing suggestion, discussing a pain study from his lab: "Testing mice in an apparatus featuring a 'free' mouse traversing an alleyway with 'jailed' mice at both ends, we observed that free female (but not male) mice would approach rather than avoid their jailed cage mates (but not strangers) if the jailed mice were in pain, spending significantly more time in proximity to a cage mate injected with acetic acid than with an uninjected mouse. This social approach to pain seemed to be analgesic, in that the duration of physical contact was inversely correlated with pain behavior of the jailed mouse."[75] One rat study also obtained results like these.[60] Consequently, some researchers have proposed that "it would be interesting to examine whether and how consolation behaviors may contribute to social buffering and whether differences among dyads in the occurrence of consolation behaviors predict differences in social buffering."[55]

Reducing conditioned fear by modifying the CS. The last process potentially at work in exposure-type studies using conditioned fear is that the presence of associates effectively modifies a previously conditioned CS, thence reducing a subject's conditioned response. Thus, for animals who were alone when learning a cue-shock pairing (for example, a tone-shock pairing), but then receive the cue with an associate (a novel combination), the presence of the associate may reduce their tendency to treat the cue as the learned CS, reducing their expectation of harm. An illustration of how such effects work is available in a study in which mice were fear conditioned to a chamber.[33] They were then tested there for fear responses, with or without the presence of a novel object (a 50 mL conical tube). The novel object was found to reduce their conditioned fear of the chamber. Superficially, this resembled how an associate might have the same effect in a conventional social buffering experience. However, the stress reduction described in this study[33] was not due to distraction or a genuine buffering effect: when the mouse was faced with an *unconditioned* challenge (an open field), the same item did not reduce stress.[33] Thus, in a conditioned fear situation, adding a new element to the frightening situation, be it an associate or even an inanimate object, can modify the CS and so reduce subjects' expectation of harm.

Implications for laboratory animal welfare

Social buffering can clearly have powerful benefits for stressed subjects. In this final section, we summarize how this phenomenon can be applied to refine research practices across diverse disciplines: important given increasing interest in and concern for laboratory animal welfare.[95] Although acting in diverse ways and dependent on species, sex, and stage of development, findings regarding buffering appear to offer viable

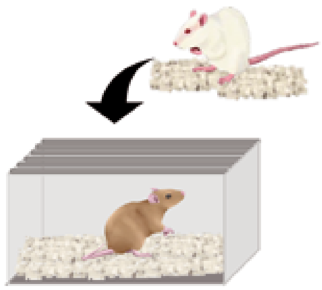
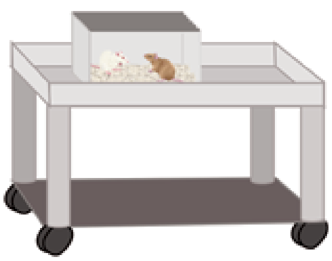
Ways to use social buffering to reduce stress in rodents	Why?	Best practice?	Plan B, if best practice is impossible?
Use social housing 	• Permits "housing-type" buffering (e.g., resilience even when outside the cage) • Avoids chronic isolation stress	• Ensure cagemates are familiar and non-aggressive • Ensure cagemates are not all stressed (e.g., not experiencing procedures) • Also give subjects company *during* stressful procedures (see "exposure-type" buffering below)	• Odors or auditory stimuli from low stress, affiliative conspecifics can partially substitute for full social contact 
Pair animals during stressful procedures outside the home cage 	• Permits "exposure-type" buffering • Avoids acute isolation stress	• Ensure associate is familiar and non aggressive • Ensure associate is not stressed (e.g., is well-habituated)	• Odors or auditory stimuli from low stress, affiliative conspecifics can partially substitute for full social contact • Artificial proxies (e.g., "tickling" by humans, for rats) can help too
Use "surprise" companions to help extinguish conditioned fear 	• Subjects exposed to aversive stimuli during or after particular cues (e.g., being wheeled on a trolley) may come to fear those cues • Adding an associate modifies the context, so reducing the subject's expectation of harm • This also permits exposure-type buffering	• Ensure associate is familiar and non-aggressive • Ensure associate is not stressed (e.g., is well-habituated)	• "Surprise" novel objects can also mitigate fear by modifying the context (but provide no additional buffering effect)

Figure 2. Overview of how social buffering can be used to improve rodent welfare during aversive husbandry and research procedures.

methods for reducing stress in all research rodents, with the possible exception of male mice presented with other males as associates. Here we suggest ways in which rodent welfare could potentially be improved based on the information this work has provided (with Figure 2 providing a simplified overview). In some cases, the practical implications of social buffering for rodent care are rather clear. In others, the data suggest new hypotheses that could be tested in future welfare-oriented research.

First, for any research rodents who are repeatedly exposed on their own to aversive stimuli in predictable ways, such that cues such as individual researchers, scrub colors, or rooms become negative CS, social buffering research suggests that providing associates to these animals, when they normally would not have company, can reduce the fear conditioning. As outlined above, this can even be accomplished by the presence of unexpected novel objects, which can similarly reduce learned fear to the conditioned context.[33] We propose that this principle could be developed into strategies to desensitize rodents who have become hyper-reactive after a series of past aversive experiences, helping to extinguish their learned fear.

A more important and immediately applicable implication of buffering research is that social housing can be used to mitigate the aversiveness of stressful procedures, even in studies that are not related to social buffering. Social housing is already recommended to increase home cage welfare and meets the needs that many rodents have for social interaction.[11,35] However, social buffering research shows that social housing *also* promotes resilience, enhancing coping abilities even for animals removed from this home environment. Physical enrichment is already known confer resilience, and although whether these and social buffering effects are additive is not yet known, it has been suggested that "multiple partners and possibly the whole socio-spatial environment" together increase resilience.[41] Social housing can thus mitigate the effects of research stressors on rats: in terms of heart rate and blood pressure, socially housed rats were less reactive to both standard research procedures like injections and to standard husbandry activities like cage-changes.[84-86] We therefore recommend that whenever research rodents are scheduled to experience aversive research procedures (for example rotarod tests, burns, injections, gavages, surgery), their homecage environments should contain high levels of social and physical enrichment to enhance their abilities to cope. Perhaps IACUCs and ACCs should ensure that rodents exposed to D or E category procedures are always provided with abundant social and physical 'enrichment', as a refinement for reducing the negative effects of aversive procedures and facilitating recovery.

Social buffering research also demonstrates that the home cage social groups best able to confer resilience are affiliative, and comprised of familiar, non-agonistic, low stress conspecifics. For some species or stages of development, this should be a specific attachment or bonded figures (that is, a mother or monogamous mates), while for others (for example, rats), they can comprise several companions, with more associates potentially conferring a greater degree of buffering. Socially-housing non-breeding male mice may be the biggest challenge because male-male aggression can be problematic in some strains, and because male mice are not always buffered by low-stress male associates.[12,51,67] However, creative methods can be used to avoid aggression in male groups (for example, transferring used nesting at cage-cleaning, and avoiding discrete enrichments that trigger resource-defense aggression), and social housing can be achieved by housing males with ovarectomized females.[26,50,93]

That low-stress affiliates are more effective as buffers leads to a further recommendation: the cage mates of research rodents exposed to distressing procedures should *not* be exposed to these procedures, so that they can remain calm and hence able to reduce stress in affected animals. Based on this finding, we further suggest that physical and social enrichment could have synergistic effects on resilience because compared with conventionally housed animals, enriched rodents are typically more affiliative and less prone to adverse behaviors like barbering or aggression.[1,6,78] This potentially makes them more effective as social buffers (although this hypothesis has not yet been tested).

Exposure-type buffering can also be an effective way to reduce stress responses to challenges. The practical implications of this are that rodents exposed to stressors outside the cage will be less adversely affected if a companion is present. The exposure-type social buffering research we reviewed on guinea pigs, rats and mice demonstrates that if subjects must be placed in a novel location (for example, for a research procedure, weighing, or myriad other reasons), their behavioral and physiologic reactions will be blunted by the presence of a conspecific. Given that housing- and exposure-type buffering effects can synergize, and given that low stress familiar associates are most likely to be effective, we again suggest that the best buffers here are non-stressed, non-research-subject cage mates. We also recommend using different animals as buffers on a rotating basis, because they may themselves be stressed by the experience due to emotional contagion. Thus, while appeasing cues from a naïve animal (for example, odors from a calm animal or affiliative physical interactions) can buffer fear in a frightened one, alarm cues from the frightened subject can also elicit fear in the naïve one, potentially reducing both the welfare of the associates *and* their effectiveness as buffers.[12,76]

If providing full social contact is impractical, then social buffering research further reveals that isolated cues from conspecifics may also be effective, albeit not quite as powerful. Furthermore, even mimicking these artificially may work. For example, social odors may reduce stress: one study found that Fos expression in the PVN was induced by placing mice in a novel test box, but this induction was reduced if other mice had previously soiled the box.[64] A rat study found similar effects from tactile cues: stress-related ultrasonic calls by pups were buffered not only by an anesthetized littermate, but also by a small object wrapped in synthetic fur or by a small piece of synthetic fur.[48] Consistent with this use of proxies, recent work shows that mimicking rat play by "tickling" with the human hand can also reduce rat stress, including their reactions to procedures like injections.[16] More research is needed to identify which cues or proxies work best depending on species, sex, and age. These cues should come from, or mimic, conspecifics who are under low stress (or even experiencing positive affective states), to be the most effective buffers.

Conclusions

To date, social buffering research has used hundreds of rodents, with hundreds more used to study the social contagion of pain and the effects of isolation stress. This work, though valuable for biological knowledge, has come at a cost to rodents themselves: many have been isolated, and exposed to challenges like shocks, wounding, and frightening novel environments. We urge that the information accrued through this work now be translated into refined husbandry and management practices that benefit the very species generating these data. The evidence shows that use of social buffering could effectively and practically enhance welfare, and refine the impact of procedures. This knowledge could therefore improve well-being for many millions of laboratory rats, mice and other research rodents.

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