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Sharing stressful experiences attenuates anxiety-related cognitive and sleep impairments

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Abstract

Anxiety is a growing public health concern that has been shown to impair both sleep and learning, and these associations have been extensively studied in recent years. In the rodent model, oftentimes various foot-shock paradigms are employed to induce stress, and subsequent sleep recordings and/or learning task results are analyzed. Previous studies have focused primarily on an individual animal's response to stress following individual stressor exposure, thereby emulating only an isolated condition. The goal of this study was to investigate the effects of socialization on stress response, and the resultant effects on sleep architecture and aversive learning. A pair-housing/pair-exposure paradigm was utilized, and the effects of unavoidable foot-shock-induced stress on sleep architecture and aversive learning were examined. The results of the present study indicate a large, positive impact of cohabitation and shared stressful experience, as rats failed to develop sleep disturbances or learning deficits. While these results indicate the benefits and importance of companionship, the underlying mechanism of this phenomenon is yet to be elucidated.

Keywords

Anxiety; Sleep; Learning; Shock-induced aggression; Social buffering; Rat

1. Introduction

Stress and anxiety are serious issues that have negative, long-lasting effects on individual physical and mental health, as well as costly implications to society as a whole [1, 2]. Anxiety and its related disorders are responsible for considerable financial and productivity

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Disclosure/conflict of interest

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losses [1]. A vast number of studies have suggested that stress and anxiety are linked to a variety of mental health disorders and neurodegenerative diseases, and that even low levels can trigger comorbidities including depression and insomnia [3–8]. In animal models, a single brief exposure to social stress can elicit gradual but chronic behavioral changes, characteristic of depression and post-traumatic stress disorder [9–15], and can also induce long-term changes in the neuroendocrine system [12, 16–22]. Additionally, previous studies have indicated that social anxiety and stress may be linked to drug abuse, as defeated male rats developed self-administration cocaine habits twice as quickly as non-defeated males [23]. It has also been shown that stressful and traumatic events are often followed by persistent disturbances in sleep, and these sleep disturbances may be predictive of future psychiatric pathologies [24, 25]. Alternatively, problems initiating and maintaining sleep may trigger anxiety disorders [24, 26, 27]. Interestingly, both insomnia and hypersomnia are common complaints of patients with anxiety disorders [27–29].

To study the immediate effects of anxiety and stress, many researchers utilize the rodent model as a convenient method for analyzing behavioral and pharmacological responses to stress. One of the most popular methods is fear conditioning training associated with stress induced by unavoidable foot-shock [15, 30–34]. Animals used to study anxiety and stress, using this type of unavoidable foot-shock, were housed and exposed to foot-shock individually [7–9, 14, 15, 30–36]. This type of animal model is ideal to measure acute responses to a foot-shock stressor by emulating a condition of an individual living in isolation and facing a stressor alone. In reality, however, socialization and cohabitation are common, and the likelihood of people encountering stressful experiences together is far greater, in which case they are likely to respond differently from isolated individuals. In order to study the effects of stress in this type of social condition, experimental animals should be housed and foot-shocked in pairs. To our knowledge, no published study has used this paired-subject model to describe the effects of foot-shock-induced stress on an individual's sleep architecture or capacity for aversive learning.

A different but equally popular rodent model to study stress and anxiety is the resident-intruder paradigm in which two male rodents are placed into a single cage and allowed to fight for a predetermined amount of time [13, 37, 38]. Such an encounter is known as “social defeat” and the winner is generally referred to as the dominant animal; the loser is referred to as the subordinate. Unfortunately, this type of behavior is not exclusive to animals, and in fact humans sometimes also use aggression to establish their social dominance [39]. More importantly, this type of aggressive encounter, in humans, occurs not only between strangers, but also between siblings, close friends, life partners, and neighbors. Aggression has also been linked to numerous psychiatric disorders, including autism, schizophrenia, affective disorders, and suicidal behaviors [40–44]. To our knowledge, no published study has systematically examined the effects of aggressive encounters on the sleep-wake activity or learning ability in humans or animals.

The present study, for the first time, examines the immediate effects of cohabitation, stressor exposure, and stressor-induced aggressive encounters on sleep-wake architecture, contextual fear memory, and avoidance learning in the rat model. Both dominant and subordinate members of a socially-housed pair were tested, to determine whether social rank has any effect on the aforementioned parameters. In this study, dominant and subordinate rats were housed in a single cage and also received foot-shock together. The results of the present study have been compared with our recently published studies which used an identical experimental protocol, except in those two studies animals were single-housed and individually-exposed [30, 32]. Compared to single-housed, individually-exposed animals, this study shows that when two rats living together are subjected to a foot-shock stressor and incited to aggression, these rats exhibit normal sleep-wake architecture, contextual memory,

and avoidance learning ability. These results, for the first time, indicate that social support during stressful experiences may have an attenuating effect on the susceptibility to sleep disturbances and learning impairments.

2. Materials and methods

2.1. Subjects and housing

Experiments were performed on 20 adult male Wistar rats (Charles River Laboratories, Wilmington, MA) weighing between 350 and 450 g. The rats were housed in pairs (two animals per cage) at 24°C with *ad libitum* access to food and water. A 12-h light/dark cycle was maintained, with lights on from 7:00–19:00h and darkness from 19:00–7:00h. The principles for the care and use of laboratory animals in research, as outlined by the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (1996), were strictly followed. Additional efforts were made to ensure that any potential discomfort, as well as the number of animals used, was minimized. To reduce additional stress that might be imposed by experimental handling, animals were handled at least once daily, beginning with the animals' arrival to the lab and continuing through the duration of the experiment.

2.2. Surgical procedures for electrode implantation

All surgical procedures were performed stereotaxically under aseptic conditions, in accordance with guidelines approved by the Institutional Animal Care and Use Committee (protocol AN-14829). Animals were anesthetized with sodium pentobarbital (40 mg/kg, i.p.; Ovation Pharmaceuticals, Deerfield, IL), mounted on the stereotaxic apparatus, and secured with blunt ear bars as previously described [45, 46]. A proper plane of anesthesia was maintained, judging by the absence of palpebral reflex or response to tail pinch. After the initial incision, the skull surface was cleaned in preparation of surgical procedure. Buprenorphine (0.03 mg/kg, i.m.; Bedford Laboratories, Bedford, OH) was administered to minimize postoperative pain.

To record states of sleep and wake, cortical electroencephalogram (EEG), dorsal neck muscle electromyogram (EMG), and hippocampal EEG (to record theta-wave) recording electrodes were chronically implanted as described previously [45, 47]. Dental acrylic was used to adhere electrodes to the skull. Conductive mini-connector pins were crimped to the end of each electrode, and collected in a plastic hub (PlasticsOne, Roanoke, VA). Immediately after surgery, animals were placed individually in recovery cages and were monitored for successful recovery, as determined by the return to consciousness, normal postures and movement, and grooming behavior.

2.3. Contextual fear conditioning and memory testing apparatus and procedures

Animals were habituated for two consecutive days, each consisting of 5 minutes of exposure to an automated freezing behavior testing (FBT) chamber (30 cm × 24 cm × 21 cm; Standard modular test chamber, ENV-008FC; Sound-attenuating cubicle, NIR-022MD; Med Associates, St. Albans, VT) made of aluminum and Plexiglas. Details of this FBT apparatus have been described in an earlier publication [30]. In the present study, the contextual fear conditioning model was used to incite foot-shock-induced aggression (SIA) behavior. After 2 habituation days, animals were placed individually and sequentially into the FBT chamber for a period of 5 minutes to measure baseline percentage of time spent in freezing, as described in our earlier publication [30]. The day after baseline freezing behavior testing, two rats that had been housed together as cage mates were placed together into the FBT chamber for a total period of 15 minutes to elicit SIA behavior. For the first three minutes, in the absence of foot-shock, spontaneous episodes of allogrooming, biting, wrestling and other affiliative behaviors were observed, in order to identify expressed dominant or subordinate

roles. After this 3-min period, to incite aggression, animals were subjected to 5 to 10 unannounced foot-shocks (1.0 mA, 1.0 s each, variable intervals). During these aggressive encounters, successful pins and intimidating/cowering postures were quantified for each animal. Based on behavioral expressions of dominant and subordinate roles, both from the non-shock and shock periods, each animal was identified as either dominant or subordinate, as described elsewhere [48–54].

On the following day of experimental testing, animals were returned to the FBT chamber. They were placed individually and sequentially into the FBT chamber for a period of 5 minutes to measure average motion and percentage of time spent in freezing. These average motion and freezing values were used as indicators of SIA fear memory. Increased percentage of time spent freezing value was indicative of contextual fear memory [30, 55].

2.4. Two-way active avoidance learning behavior

A two-way active avoidance (TWAA) paradigm was utilized to measure avoidance learning, as described in detail in our earlier publications [47, 56, 57]. On the day after animals experienced shock-induced aggression, and immediately following fear memory testing, animals were placed into the TWAA apparatus. The experimental procedures were administered on separate, subsequent days in order to minimize the effect that exposure to inescapable foot-shock would have on TWAA learning, which uses escapable foot-shock. The procedure involved first placing rats individually into one compartment of the TWAA apparatus. Following a 2 min acclimatization period, animals were subjected to a series of 15 TWAA trials. The procedures for the conditioned stimulus (CS) and unconditioned stimulus (UCS) were as follows: A tone (3600 Hz; 45 dB) and a pulsatile light (2.5 Hz) were presented as a CS in the compartment with the animal, and 10 s later a scrambled foot-shock (1.0 mA; 5 s) was delivered through the floor grid as UCS. To avoid receiving a foot-shock, the rat had 10 s to move to the opposite compartment. If the animal did not move to the other compartment, UCS was delivered for a maximum of 5 s, and CS ended with UCS. While receiving UCS, if the animal moved to the other compartment, both CS and UCS ended immediately. The inter-trial interval was fixed at 60 s. The percentage of trials in which the animals successfully avoided UCS was collected and used as an indicator of avoidance learning.

2.5. Determination of sleep-wake behavioral states

Immediately following SIA, animals were connected to a polygraphic recording system. For the purpose of determining possible effects of SIA on sleep-wake states, polygraphic data were captured over a 6-h period (between 10:00–16:00h) with a computer using Gamma software (Grass product group; Astro-Med, West Warwick, RI). From these captured data, three behavioral states (wakefulness: W, slow-wave sleep: SWS, and REM sleep) were distinguished and scored visually using Sleep Sign for Animal (Kissei Comtec Co., Ltd., Tokyo, Japan). The physiological criteria for the identification of these wake-sleep states were described in our earlier publications [56, 58]. The percentage of time spent in W, SWS, and REM sleep, the latencies to the onset of SWS and REM sleep, and the total number of REM sleep episodes were quantified for each animal.

2.6. Determination of social rank (dominant and subordinate)

To determine the role, if any, that social rank plays on sleep architecture and learning ability, the animals in each pair were assigned either a dominant or subordinate designation. Once the experiments were complete, three separate investigators reviewed the video recordings of SIA sessions. For each video, any actions indicative of dominance, as well as the times at which these behaviors occurred, were noted for both animals. All instances of dominant behavior were evaluated according to a predetermined list of criteria. Investigators

were trained to pay careful attention to four events of interest: (1) play fighting, defined as normal wrestling behavior prior to shock administration [49, 50, 59]; (2) affiliative behavior, in which one animal follows the other around the cage enclosure [50, 53]; (3) allogrooming, which occurs when one animal actively grooms the other, particularly around the neck and abdomen [54]; and (4) aggressive encounters, in which one animal successfully pins or dominates the other [51, 52] as the result of a shock administration.

The separate incidents were then tallied, and experimenters determined which of the pair was the dominant animal and which was subordinate by comparing the total number of dominant behavior instances for each animal. In the one case where experimenters could not reach a consensus as to the social rank of a pair of animals, the video was submitted for a second round of double-blind evaluation. All three investigators assessed a series of three videos, unaware as to which video was the source of disagreement. The inter-rater reliability for this phase of assessment was 95.24%.

2.7. Statistical analysis

All data were expressed as the mean $\pm$ standard error of means (SEM). Statistical significance was determined by one or two-way analysis of variance (ANOVA). Bonferroni posttests were used for post-hoc comparisons. All of these statistical tests were performed using Prism software (GraphPad Software, Inc., La Jolla, CA). Individual statistics for specific comparisons are described in the results section.

3. Results

A total of 6 pairs of rats expressed SIA behavior. Based on the behavioral criteria described above, one rat within each pair was labeled dominant and the other rat was labeled subordinate. Within each pair, the dominant animal was slightly heavier (2% – 3% of total body weight) than its subordinate cage mate. However, the mean body weight of the dominant group was not significantly different than the mean body weight of the subordinate group (t-test; df = 5; $t = 1.33$; $p = 0.24$). The following results describe the effects of SIA on the sleep-wake activity, contextual fear memory, and avoidance learning in the dominant and subordinate groups.

3.1. Effects on sleep-wake activity

The changes in the sleep-wake parameters are summarized in Figure 1. The total percentages of time spent in W, SWS, and REM sleep, as well as sleep latency, REM sleep latency, and total number of REM sleep episodes during the baseline recording session (before SIA, between 10:00h–16:00h) were not significantly different (two-way ANOVA and Bonferroni posttest) between the dominant and subordinate groups. Thus, under baseline recording conditions, before being subjected to a session of SIA, the dominant and subordinate groups were initially equal in terms of sleep-wake architecture and time spent in W, SWS, and REM sleep.

3.1.1. Effects of SIA on wakefulness—Changes in the total percentages of time spent in W are summarized in Figure 1A. In the dominant group, the total percentages of time spent in W were not significantly different (Bonferroni posttest) between the baseline recording session and the recording session immediately after expression of SIA (Fig. 1A). Like the dominant group, the subordinate group total percentages of time spent in W were not significantly different between the baseline recording session and recording session immediately after expression of SIA (Fig. 1A). These results indicate that in both the dominant and subordinate groups, SIA did not change the total percentages of time spent in W. Similarly, post-hoc tests (Bonferroni posttest) comparing the dominant and subordinate

groups revealed that the total percentages of time spent in W after a session of SIA were not significantly different (Fig. 1A). These results indicate that after SIA, the dominant rats were not different than the subordinate rats in relation to total percentages of time spent in W.

3.1.2. Effects on SWS—Changes in the total percentages of time spent in SWS are summarized in Figure 1B. In the dominant group, the total percentages of time spent in SWS were not significantly different (Bonferroni posttest) between the baseline recording session and recording session after SIA (Fig. 1B). Similarly, in the subordinate group, the total percentages of time spent in SWS were not significantly different between the baseline recording session and the recording session after expression of SIA (Fig. 1B). These results indicate that SIA did not change the total percentages of time spent in SWS. Similar post-hoc tests revealed that the total percentages of time spent in SWS after SIA were also not significantly different between the dominant and subordinate groups (Fig. 1B). In addition to the total percentages of time spent in SWS, sleep latencies of both the dominant and subordinate groups were not significantly different (one-way ANOVA) between the baseline and post-SIA recording sessions (Fig. 1D). Similarly, sleep latencies after SIA were not significantly different between dominant and subordinate groups (Fig. 1D). These results indicated that after SIA, the dominant rats were not different than the subordinate rats in relation to the total percentages of time spent in SWS and sleep latency.

3.1.3. Effects on REM sleep—Changes in the total percentages of time spent in REM sleep are summarized in Figure 1C. In the dominant group, the total percentages of time spent in REM sleep were not significantly different (two-way ANOVA) between the baseline recording session and the recording session after SIA expression (Fig. 1C). Like the dominant group, in the subordinate group, the total percentages of time spent in REM sleep were not significantly different between the baseline recording session and recording after SIA expression (Fig. 1C). These results indicate that SIA did not change the total percentages of time spent in REM sleep. The total percentages of REM sleep after SIA were also not significantly different between the dominant and subordinate groups (Fig. 1C). In addition to the total percentages of time spent in REM sleep, the REM sleep latencies of both dominant and subordinate groups were not significantly different (one-way ANOVA) between baseline recording sessions and recording sessions after SIA (Fig. 1E). Similarly, REM sleep latencies after SIA were not significantly different between the dominant and subordinate groups (Fig. 1E). Like the total percentages of time spent in REM sleep and REM sleep latencies, the total number of REM sleep episodes was not significantly different (one-way ANOVA) between the baseline recording session and recording session after SIA (Fig. 1F). Similarly, the total number of REM sleep episodes during the recording sessions after SIA was not significantly different between the dominant and subordinate groups (Fig. 1F). These results indicate that after SIA, the dominant group of rats was not different than the subordinate group of rats in relation to total percentage of time spent in REM sleep, REM sleep latency, and total number of REM sleep episodes.

3.2. Contextual fear memory

To assess contextual fear memory, the average motion and the percentage of time spent freezing of each animal was analyzed. Changes in the average motion and freeze response in the dominant and subordinate groups are summarized in Figure 2. During the baseline freezing behavior recording day, both average motion and the total percentage of time spent in freezing were not significantly different (one-way ANOVA) between the dominant and subordinate groups (Fig. 2A and 2B). These results indicate that before exposure to SIA protocol, these groups expressed similar levels of activity and freezing. Specifically, both the dominant and subordinate groups demonstrated high activity (Fig. 2A) and very little freezing behavior (Fig. 2B) prior to SIA exposure. On the contrary, on the contextual fear

memory testing day, both dominant and subordinate groups exhibited high levels of freezing behavior (Fig. 2B) and decreased average motion (Fig. 2A). Similar to the baseline freezing behavior recording day, there was no significant group variation (one-way ANOVA) in average motion or in the percentage of time spent freezing during the recording session after SIA expression. These results indicate that the level of contextual fear memory was comparable between the dominant and subordinate groups.

It is worth mentioning that the experimenters noted a change in the type of motion behavior exhibited in the FBT chamber in the animals on the days before and after SIA. Before SIA exposure (baseline day), the animals demonstrated normal grooming and explorative behaviors, however, on the day after SIA exposure, the behaviors were indicative of the animals' desire to escape the FBT chamber (i.e. scratching at the edges and corners of the box). These observations support our belief that the rats were demonstrating genuine fear as a result of contextual fear memory associated with the FBT chamber.

3.3. Avoidance Learning

The differences in avoidance learning between the dominant and subordinate groups, as well as an established control group, are summarized in Figure 2C. The control group included 6 pairs of animals with equivalent age, weight, and sex characteristics to those used in the experimental groups. Control animals were pair housed, but did not undergo shock-induced aggression. The subordinate group of animals exhibited a tendency to avoid foot-shock more frequently ($25.90 \pm 6.52\%$) than the dominant group of animals ($17.87 \pm 6.23\%$). The established control group demonstrated the highest capacity for two-way active avoidance learning ($29.98 \pm 9.23\%$). However, these differences were not statistically significant (one-way ANOVA). These results indicate that dominant rats are not different than subordinate rats in capacity for avoidance learning. Additionally, the results suggest that expression of SIA bears little if any effect on an animal's ability to learn a two-way active avoidance task.

4. Discussion

The principal findings of this study demonstrate that animals assuming a dominant or a subordinate role develop no significant differences in sleep architecture, fear memory or aversive learning after exposure to fear-inducing foot-shock. Shock-induced aggressive stimulus had no significant effect on sleep architecture in either experimental group. Furthermore, shock-induced aggression did not affect the animals' ability for aversive learning, as compared to non-shocked control animals. The present study suggests, for the first time, that sharing social experiences can attenuate fear acquisition following an aversive stimulus, while learning capacity remains unaffected.

Previously, research using social defeat and contextual fear conditioning via foot-shock has been primarily focused on individual animal responses. Animals were housed individually, and encountered each other only during times of social confrontation [13, 37, 38], or else never interacted and encountered foot-shock-induced contextual fear separately and individually [15, 30–34]. The main advantage of this foot-shock model is the ability to measure the acquisition, expression, and extinction of stress [7, 15, 30, 32, 34]. This contextual fear conditioning model, based on classical conditioning, can additionally be used to study memory acquisition, consolidation, and retrieval in rodents [55, 60, 61]. Alternative paradigms may use a variety of stressors, such as immobilization, corticosterone administration and open field exposure, though such techniques bear a considerable effect on sleep-wake behavior and produce unpredictable and often unknown levels of stress in subjects [30, 62–64]. Relatively few studies investigated the role of socialization on contextual fear learning [65–68], and, to our knowledge, no study has yet explored fear memory, aversive learning, and sleep as they are affected by shared social experiences.

Before exposure to shock-induced aggression, both the dominant and subordinate groups demonstrated comparable levels of average motion and comparable freezing behavior. Together, these findings suggest the validity of the model used in our study. That is, prior to experiencing a stressful shock stimulus, animals that have been housed together show no significant differences in anxiety-like behavior. The fact that the two groups showed similar average motion and freeze behavior after SIA exposure only strengthens the internal validity of the paired model. Additionally, the absence of any changes in sleep architecture that occur after SIA exposure further supports the use of the paired-housing model. Together, these behavioral and physiological consistencies across experimental treatments indicate that dominance roles were not an issue that affected results.

Members of both the dominant and subordinate groups reacted similarly to a stressful experience. Specifically, both groups demonstrated an increase in anxiety-like behavior (less average motion and higher % time freezing) after shock exposure. The increase in these anxiety-like behaviors was attenuated both by the animals' cohabitation and their shared exposure to a negative shock stimulus [69]. It is difficult to determine exactly how much of an influence sharing the negative stimulus had on each group's behavior. Animals that are housed together outside of experimental testing show highly variable behavioral responses to shock stimulus, as compared to animals housed separately [67]. Additionally, studies have found that social housing following an episode of social defeat prevents the following increase in anxiety-related behaviors in defeated animals [70, 71], suggesting that dominant and subordinate group animals are differentially affected by socialization.

The social support that follows a stressful experience affects a variety of behavioral and cognitive functions. In the present study, animals exposed to SIA demonstrated no impaired capacity for procedural learning on the two-way active avoidance task. Von Frijtag and colleagues [72] found that animals housed individually after defeat exhibit impairments to cognitive processes, including the ability to anticipate a reward, but that social housing prevented these long-term cognitive deficiencies.

This phenomenon is known as "social buffering" and has been studied in depth in recent years [69, 73–75]. In the animal model, visual and olfactory exposure to familiar conspecifics is not enough to provide the benefits of social buffering; on the contrary full physical contact is necessary for social interaction to attenuate the negative effects of a stressful experience [75]. Rats are the most commonly studied model for this type of social effect, though similar findings have been reproduced with mice, calves, and even primates [76–79]. Additionally, the cognitive impairments that are mitigated by social support have been likened to human anhedonia, which is a symptom characteristic of clinical depression [72].

Because it is known that cohabitant animals require many exposures to an aversive stimulus for response to stabilize [66], future research on social buffering may attempt multiple SIA sessions for each pair. Numerous studies have revealed physical changes in brain chemistry that are caused by socialization, and which are not observed in animals living in isolation [80–83]; however, countless biochemical and neurological changes resulting from cohabitation still remain to be identified.

5. Conclusion

The present study indicates that social buffering may be a useful tool for mitigating the anxiogenic effects of a stressful experience, while leaving cognitive abilities unimpaired. The findings of this animal study have important implications, and may impact clinical research ranging from panic attacks to post-traumatic stress disorder. The use of

cohabitation as a clinical tool provides an alternative or a supplementary treatment for myriad psychiatric afflictions, and may provide a framework for understanding how people deal with anxiety.

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Figure 1.

Effects of shock-induced aggression stressor on sleep-wake architecture. Bars in the left-hand column represent percentages (mean + SE) of (A) time spent in wakefulness, (B) time spent in slow-wave sleep (SWS), and (C) time spent in rapid eye movement (REM) sleep, for both dominant and subordinate groups during each of the 6-h periods following baseline and SIA treatments. Bars in the right-hand column represent total (mean + SE) (D) latency to SWS onset, (E) latency to REM sleep onset, and (F) number of REM sleep episodes, for both D and S groups during each of the 6-h periods following baseline and SIA treatments. Key: D-BL, dominant baseline; S-BL, subordinate baseline; D-SIA, dominant shock-induced aggression; S-SIA, subordinate shock-induced aggression.

Figure 2.

Effects of shock-induced aggression stressor on (A) average motion, (B) percentage of time spent freezing, and (C) percentage of successful avoidances in the TWAA task. Bars represent totals or percentages (mean + SE) for both dominant and subordinate groups during baseline and following SIA treatments. Key: D-BL, dominant baseline; S-BL, subordinate baseline; D-SIA, dominant shock-induced aggression; S-SIA, subordinate shock-induced aggression; C-SIA, control for shock-induced aggression. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.