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Review

Behaviorally inhibited individuals demonstrate significantly enhanced conditioned response acquisition under non-optimal learning conditions

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HIGHLIGHTS

- Behavioral inhibition is a known risk factor for anxiety disorder development.
- Inhibited individuals demonstrate enhanced conditioned eyeblink response acquisition.
- Enhancements were greater under non-optimal relative to full-reinforcement paradigms.
- Motivational aspects of uncertainty or general potentiation may influence etiology in BI.

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ABSTRACT

Behavioral inhibition (BI) is an anxiety vulnerability factor associated with hypervigilance to novel stimuli, threat, and ambiguous cues. The progression from anxiety risk to a clinical disorder is unknown, although the acquisition of defensive learning and avoidance may be a critical feature. As the expression of avoidance is also central to anxiety development, the present study examined avoidance acquisition as a function of inhibited temperament using classical eyeblink conditioning. Individuals were classified as behaviorally inhibited (BI) or non-inhibited (NI) based on combined scores from the Adult and Retrospective Measures of Behavioural Inhibition (AMBI and RMBI, respectively). Acquisition was assessed using delay, omission, or yoked conditioning schedules of reinforcement. Omission training was identical to delay, except that the emission of an eyeblink conditioned response (CR) resulted in omission of the unconditioned airpuff stimulus (US) on that trial. Each subject in the yoked group was matched on total BI score to a subject in the omission group, and received the same schedule of CS and US delivery, resulting in a partial reinforcement training schedule. Delay conditioning elicited significantly more CRs compared to the omission and yoked contingencies, the latter two of which did not differ from each other. Thus, acquisition of an avoidance response was not apparent. BI individuals demonstrated enhanced acquisition overall, while partial reinforcement training significantly distinguished between BI and NI groups. Enhanced learning in BI may be a function of an increased defensive learning capacity, or sensitivity to uncertainty. Further work examining the influence of BI on learning acquisition is important for understanding individual differences in disorder etiology in anxiety vulnerable cohorts.
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1. Introduction

Avoidance is a defining feature of behavioral inhibition (BI), a risk factor for the development of anxiety disorders [1,2]. In addition to avoidance, longitudinal and cross-sectional studies of BI have identified other stable characteristics, including social reticence and enhanced reactivity to novelty, threat, and uncertainty [3–5]. The manner by which these factors influence the transition from anxiety vulnerability to a clinical disorder, however, remains obscure [6]. A possible consequence of the established BI phenotypes may be a sensitivity to associative learning acquisition. Indeed, converging evidence supports the role of classical conditioning in the etiology and maintenance of anxiety disorders [7,8]. Further evidence supporting this relationship is found in a recent study examining classical conditioning in a population of veterans tested on a classical eyeblink conditioning task [9]. Retrospective assessment of childhood BI in this veteran group was associated with enhanced conditioned response acquisition. However, the groups generally displayed low levels of conditioned responding overall as well as expressing co-morbid pathologies, which necessarily add complexity to conclusions made about learning and BI.

The finding of enhanced cue-outcome acquisition in veterans has been supported by work in an animal model of inhibited temperament. Wistar Kyoto (WKY) rats, which are an inbred, stress sensitive strain [10,11] demonstrate facilitated cue-outcome acquisition compared to an outbred rat strain [12,13]. The WKY rats were found to emit more CRs and achieve higher asymptotic performance over the conditioning sessions. In addition to these observations, WKY rats have also demonstrated enhanced response–outcome learning during an operant lever-press avoidance task [14]. As such, a sensitivity to both prediction, in cue-outcome acquisition, and control, in response–outcome acquisition is apparent in this animal model of inhibited temperament. At present, it is not known if non-clinical, behaviorally inhibited human subjects would show similar patterns of acquisition across learning paradigms.

The acquisition of avoidance in human participants has been primarily evaluated using fear conditioning paradigms, although these procedures pose some difficulty concerning discomfort from aversive stimuli. For example, in fear conditioning studies using a shock US, participants are required to set their level of tolerance to the shock intensity prior to beginning the experiment [15–17], resulting in subjective interpretation of US aversiveness. Alternatively, objective assessment of avoidance acquisition can be attained by using standard classical conditioning procedures and adding the use of an omission contingency. During omission training, the detection of a conditioned eyeblink response results in elimination of the US on that trial. In human eyeblink conditioning studies, the US most often employed is an airpuff set at a fixed pressure intensity. Subjective adjustment of US aversiveness across participants is thus eliminated. All subjects receive the same US intensity, and individual differences in reactivity – and consequences thereof – can be more clearly assessed. An added benefit of employing this classical conditioning protocol is the ability to examine avoidance acquisition without explicitly conditioning fear. Generally, avoidance and its relationship to anxiety function to impede one's ability to cope with all life events, not just explicitly fearful ones [18]. Therefore, using neutral or mildly aversive stimuli provide a better understanding about the nature of associative learning and its potential impact on risk for anxiety disorder development.

Several early human eyeblink studies have examined response–outcome learning using an omission contingency to assess the effect of instrumental US reinforcement on CR acquisition. In these studies, omission training resulted in reduced acquisition compared to delay conditioning [19,20]. Furthermore, some studies also enabled a comparison of CR acquisition in individuals given the same pattern of US exposures, but without control over US delivery [20,21]. This was achieved by adding control groups yoked to the individual reinforcement schedules of participants in the omission groups. When yoked groups were included, they generally demonstrated inferior performance relative to the omission groups, though both groups received the same number of US exposures by design [20,21]. Because of these observed response patterns, it was suggested that participants receiving omission training were acquiring an avoidance response. Lower response rates apparent in the yoked groups were likened to those observed during partial reinforcement training. With the exception of the recent study in military veterans [9], no prior work using similar protocols has examined individual differences that affect CR acquisition, such as stress reactivity, clinical anxiety, or risk for disorder development. Thus it is not known how anxiety vulnerability might influence avoidance learning or acquisition under degraded learning conditions.

In following, the present study was designed to examine eyeblink conditioning, sensitivity to avoidance acquisition, and partial reinforcement training in inhibited and non-inhibited individuals. Groups were classified as inhibited or non-inhibited based on combined scores from the adult and retrospective measures of behavioral inhibition (AMBI and RMBI, respectively), resulting in a total BI score. Conditioned response acquisition was then compared over three paradigms: standard cue-outcome learning via delay eyeblink conditioning, response–outcome learning using an omission reinforcement schedule, and a yoked control contingency. The imposition of an omission contingency during response acquisition was used to measure sensitivity to negative reinforcement and avoidance acquisition. In the yoked contingency, each participant was matched to a participant in the omission group based on their total BI score and received the same corresponding pattern of CS/US reinforcement as their matched counterpart. It was expected that acquisition during delay conditioning would exceed that of omission and yoked overall, which would be consistent with prior work. With respect to anxiety vulnerability, as BI is associated with enhanced reactivity to novel and negative stimuli as well

as avoidant personality features, it was expected that inhibited temperament might also confer a bias toward response–outcome learning during omission training. Yoked training was expected to result in the lowest rates of acquisition overall.

2. Materials and methods

2.1. Participants

One hundred nine college-aged students were recruited from the University of Northern Colorado, School of Psychology. Students voluntarily participated to receive class credit or extra credit for psychology classes. Seventy-one females and 38 males with mean age of 20.6 (SD = 4.4, range 18–45) and mean education of 13.7 years (SD = 1.5, range 12–17) were included in the study. Informed consent was obtained in accordance with procedures approved by the University of Northern Colorado Institutional Review Board.

2.2. Materials and apparatus

The eyeblink conditioning apparatus and procedures were similar to that previously described [22]. The tone stimulus was produced with Coulbourn Instruments (Allentown, PA, USA) signal generators and passed to a David Clark aviation headset (Model H10-50, Worchester, MA, USA). Sound levels were verified with a Realistic sound meter (RadioShack, Fort Worth, TX, USA). The headset was fitted with a boom capable of delivering the airpuff stimulus. The boom was placed 1 cm from the participant's eye and aimed directly at it. Air puffs were produced by pressurizing ambient air to 5.5 psi (Furgut Industries, Aitrach, Germany), and released through Silastic tubing by a computer-controlled solenoid valve (Clipper Instruments, Cincinnati, OH, USA). To transduce the eyelid EMG signal, pediatric silver/silver chloride EMG electrodes with solid gel were placed above and below the left eye, with the ground electrode placed on the neck. The EMG signal was passed to a medically isolated physiological amplifier (UFI, Morro Bay, CA, USA), low-pass filtered and amplified 10 K. The EMG signal was sampled at 500 Hz by an A/D board (PCI 6025E, National Instruments, Austin, TX, USA) connected to an IBM-compatible computer. Software control of stimulus generation was performed by LabView (National Instruments). For the omission contingency, a time-varying Gabor filter processed the data up to 10-ms prior to US trigger. If a CR was detected from 40–440-ms during CS presentation, the US trigger was not initiated.

2.3. Psychometric scales

Study participants completed the Adult Measure of Behavioural Inhibition (AMBI), the Retrospective Measure of Behavioural Inhibition (RMBI) [23], and the State/Trait Anxiety Inventory (STAI) [24]. The AMBI is a 16-item self-report inventory that assesses current tendency to respond to new stimuli with inhibition and/or avoidance, and has also been shown to be a measure of anxiety proneness. The RMBI is an 18-item self-report inventory used to assess childhood memories of exhibiting inhibition to the unfamiliar [23]. As originally published, the RMBI allows respondents to select a "do not remember" response; these responses constitute missing data for the corresponding items on analysis. To avoid this potential for data loss, a modified version of the RMBI was used, which eliminated "do not remember" as a response option.

2.4. BI group matching

Individuals were consented for a prescreening session during which time they filled out the AMBI/RMBI and STAI scales. The AMBI/RMBI scores were combined to determine a total BI score. Participants were subsequently matched on total BI in pairs. For each pair of participants, the first individual was randomly assigned to either the Delay or Omission group, and the second participant was assigned to the other group. Additionally, a yoked control group was comprised of participants individually matched to a member of the omission group using their total BI score. Each matched individual was run using a US reinforcement schedule identical to his or her corresponding omission counterpart. Attempts were made to match BI levels between all groups.

2.5. Conditioning session

Upon arrival to the study, participants were consented and instructed that the study was going to evaluate responses to tones and air puffs to the eye, that they were to watch a silent video of their choice (e.g. a nature video with sound muted), and that they were to remain awake during the testing session. Participants were then fitted with EMG electrodes and headphones, EMG signal quality was verified, and the conditioning program was started. The program began with three US-alone (50-ms, 5.5 psi airpuff) exposures to assess UR quality and magnitude for all participants. The acquisition session began immediately following the US exposures. Delay training consisted of 60 paired CS-US trials (500-ms/1200 Hz pure tone CS overlapping and co-terminating with the US), and 20 CS-alone extinction trials. For individuals in the omission group, all parameters were identical to delay training, except that the emission of a CR at least 10 ms prior to US onset resulted in removal of the US for that trial. For the yoked control group, the pattern of paired CS-US trials and CS alone (US omitted) trials was based on the pattern of CRs produced by their matched omission counterpart. The inter-trial interval varied pseudorandomly between 30 ± 5 s for all contingencies.

2.6. Signal processing and data reduction

EMG data was evaluated on a trial-by-trial basis for all participants. Processing of eyeblink responses followed methods previously reported [22,25]. To determine the occurrence of an eyeblink, EMG activity was first lowpass filtered with a Lowess filter (Stat-Sci, Tacoma, WA, USA) using a time constant of 0.025, and a smoothing interval of 5. Smoothed EMG activity was evaluated for a 500-ms window beginning at the onset of the CS as well as for a 125-ms comparator window that immediately preceded the CS window. A CR was scored when an eyeblink occurred 40-ms after CS onset but before US onset that exceeded the mean activity, plus 4 times the standard deviation, of the activity in the comparator window. A UR was scored when an eyeblink was produced 0–100-ms after US onset. Those sessions with excessive signal noise (loss of more than 10% of trials), equipment malfunction, or incomplete session data (e.g. falling asleep), were discarded and not used for further analysis. Inspection of the eyeblink conditioning session therefore resulted in rejection of data from 19 participants (Delay = 4, Omission = 5, Yoked = 10). The resulting number of participants in each group was: Delay = 30, Omission = 30, Yoked = 30.

2.7. Data analysis

To examine the main effects and interactions of anxiety vulnerability and CR acquisition, the 80 trial conditioning session was divided into 10 trial blocks and evaluated independently for acquisition (6 blocks) and extinction (2 blocks). Group designations for behaviorally inhibited and non-inhibited were established by dividing the AMBI and RMBI scales by published standards [23], resulting in approximately one-third of the participants being classified as "inhibited", and two-thirds as "non-inhibited". Results were evaluated using between group measures including Group

Table 1
Psychometric and demographic data sorted by contingency group and inhibition classification (behaviorally inhibited/BI; non-inhibited/NI). Scores are reported for total BI, which is composed of combined scores from the Adult and Retrospective Measures of Behavioural Inhibition (AMBI, RMBI, respectively). State/Trait Anxiety Inventories are also reported. BI was positively correlated with AMBI, RMBI, and State/Trait inventories ($p < .01$). None of the measures differed significantly with respect to sex or contingency group designation.

	N	BI	AMBI	RMBI	State	Trait	Acquisition %CR	Extinction %CR
Group								
Delay								
BI	11 (3 Male)	39.6 (8.6)	18.6(4.8)	21.0 (5.4)	38.1 (8.9)	40.4 (7.0)	77.6	35.0
NI	19 (10 Male)	18.6 (5.5)	9.3 (2.8)	9.3 (4.3)	31.2 (8.2)	37.7 (8.7)	69.3	29.2
Omission								
BI	10 (4 Male)	36.0 (5.7)	18.5 (4.5)	17.5 (4.8)	38.9 (7.5)	48.2 (10.7)	51.2	39.0
NI	20 (5 Male)	21.0 (5.1)	9.5 (2.9)	11.6 (4.4)	36.4 (9.8)	36.7 (8.6)	41.8	32.5
Yoked								
BI	10 (3 Male)	36.1 (5.9)	18.3 (5.7)	17.8 (5.0)	37.3 (10.6)	39.9 (10.5)	50.1	43.5
NI	20 (9 Male)	20.6 (5.6)	11.3 (4.1)	9.2 (3.2)	32.5 (8.6)	34.8 (8.1)	38.5	28.8

(Delay, Omission, Yoked), and BI (Inhibited; BI or Non-Inhibited; NI), with Block as a within subject measure. Significant effects from the ANOVAs were followed up with planned *F*-tests and Boneferroni post-hoc comparisons. The level of significance was set at a $p < .05$.

3. Results

3.1. Psychometric data

The psychometric and demographic data for the BI and NI groups are summarized in Table 1. BI scores were positively correlated with the AMBI ($r = 0.85$, $p < 0.01$) and RMBI ($r = 0.88$, $p < 0.01$), Trait ($r = 0.39$, $p < .001$), and State inventories ($r = 0.31$, $p < 0.01$). The AMBI and RMBI were also positively correlated ($r = 0.49$, $p < 0.01$), which is consistent with published standards [23].

There were no significant sex differences between groups on any of the measures, and no significant differences as a function of contingency group assignment (all *p*'s > .25).

3.2. Acquisition

Eyeblink response acquisition in group Delay was clearly higher than groups Omission and Yoked over the 6 acquisition blocks [Fig. 1]. This was confirmed with a 3 (Group) × 2 (BI) × 6 (Block) ANOVA which revealed a significant main effect of Group $F(2,84) = 13.17$, $p < .001$, while a main effect of block, $F(5,420) = 27.08$ $p < .001$, confirmed that acquisition occurred over the conditioning session. Post hoc comparisons of the group main effect reaffirmed a significant difference in total session acquisition between group Delay (73%) compared to both Omission (46%) and Yoked (44%). With respect to anxiety vulnerability, there was a significant interaction of BI × Block, $F(5,420) = 2.28$, $p < .05$, indicating that inhibited individuals demonstrated more CRs over the acquisition session that was dependent on block, and independent of reinforcement contingency [Fig. 2]. To evaluate this interaction, specific comparisons were conducted between groups BI and NI, collapsed over training condition. These comparisons indicated significantly more CRs emitted in group BI relative to NI during the last two blocks of acquisition. Further, rates of acquisition were found to plateau over blocks 2–6 for group NI, while CR percentages on blocks 5 and 6 were significantly higher than blocks 1 and 2 for group BI, indicating continued response acquisition over the training session.

Although our initial hypothesis of avoidance acquisition was not confirmed, acquisition was greater in inhibited relative to non-inhibited individuals under yoked/partial reinforcement training (BI—51%; NI—42%), as well as during omission (BI—50%; NI—39%). Therefore, as the omission and yoked groups received the same schedule of US delivery and resulted in similar rates of conditioned responding, these groups were combined to examine the relationship between inhibited temperament and acquisition under partial reinforcement training. A 2 (BI) × 6 (Block) ANOVA revealed a main effect of Block, $F(5,290) = 13.73$, $p < .001$, and an interaction of BI × Block, $F(5,290) = 2.66$, $p < .05$. Post hoc comparisons showed significantly greater CRs emitted by group BI relative to NI on blocks 5 and 6 of acquisition when receiving intermittent US reinforcement [Fig. 3].

3.3. Extinction

Analysis of extinction training using a 3 (Group) × 2 (BI) × 2 (Block) ANOVA revealed a significant interaction of BI × Block,

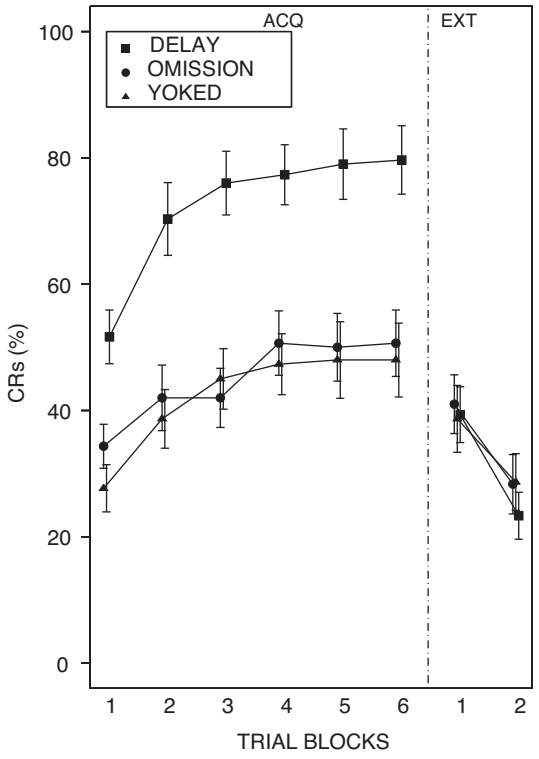

Fig. 1. Percent conditioned responses during 60 trials of acquisition (Acq) and 20 trials of extinction (Ext) in groups Delay, Omission, and Yoked. Trial blocks are denoted on the *x*-axis; each block consists of 10 trials. Percent CRs are indicated on the *y*-axis. The groups receiving Omission and Yoked training emitted significantly fewer CRs relative to group Delay. Conditioned responding did not differ during extinction. Error bars represent standard error of the mean.

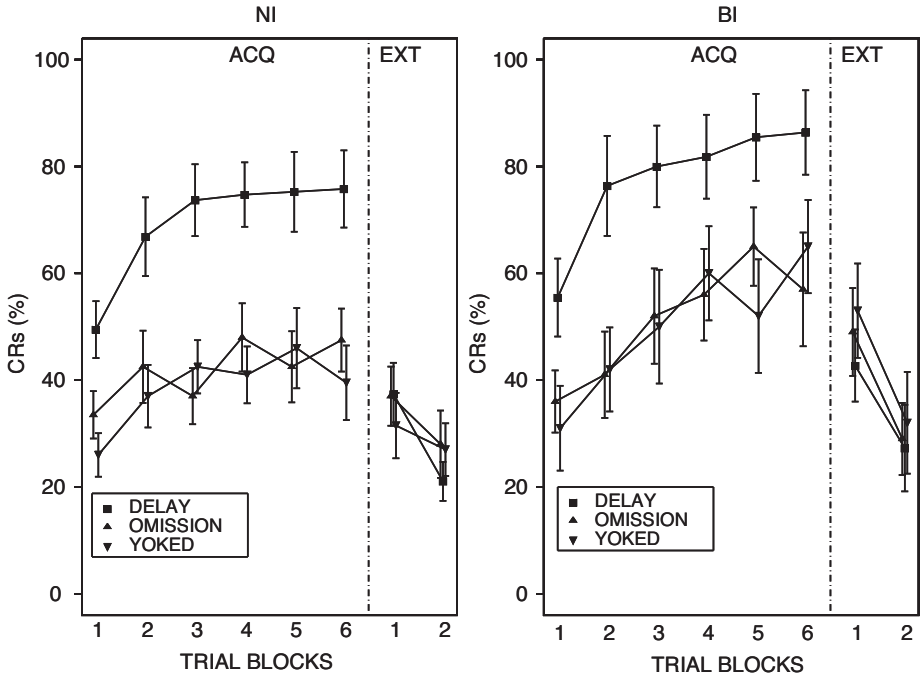

Fig. 2. Conditioned response acquisition in Delay, Omission, and Yoked groups as a function of inhibited temperament. Acquisition for non-inhibited (NI) individuals [left panel] was significantly lower overall compared to acquisition in behaviorally inhibited (BI) individuals [right panel]. Acquisition and extinction trial blocks are indicated on the *x*-axis, percent CRs are denoted on the *y*-axis. Error bars represent standard error of the mean.

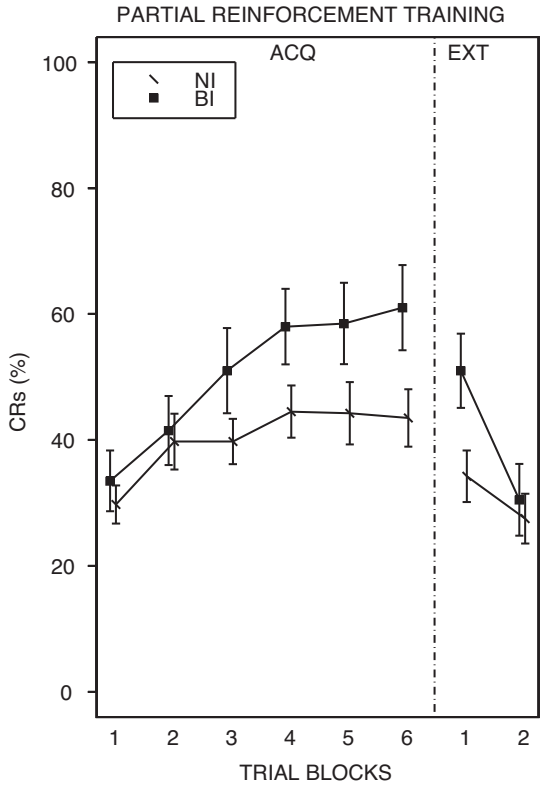

Fig. 3. Conditioned response acquisition in BI and NI groups during partial reinforcement training (combining Omission and Yoked groups which received identical schedules of paired CS/US trials). Conditioned responding in BI and NI groups was similar during the first half of the conditioning session, while group BI continued to increase CR emission as the session progressed, despite the frequency of US reinforcements decreasing. CRs were significantly greater in BI relative to NI groups on blocks 5 and 6 of acquisition. Error bars represent standard error of the mean.

$F(1,84) = 4.39$, $p < .05$. This reflected the emission of a greater percentage of CRs by group BI on block 1 of extinction compared to group NI, while responding was the same for both groups on block 2. However, as groups BI and NI had different levels of performance at the end of training, it was necessary to evaluate extinction with respect to these CR asymptotes. In following, a score for each subject was calculated by dividing CR percentages during extinction by CR percentages on acquisition trials 41–60 for each participant. A 3 (Group) × 2 (BI) ANOVA of these data revealed a non-significant trend in extinction rates between training groups, ($F(2,84) = 3.04$, $p = .06$), with conditioned responding during extinction occurring at 51%, 79%, and 95% of asymptotic performance for groups Delay, Omission, and Yoked, respectively.

3.4. Reactivity

Sensitivity in responding to the US is a factor which can potentially influence rates of CR acquisition. Therefore, UR magnitude to three US-alone presentations was assessed for each participant prior to beginning the conditioning session. Means and standard deviations for UR magnitude were 4.1 (1.6) and 3.5 (1.4) for groups BI and NI, respectively. A 3 (Group) × 2 (BI) ANOVA confirmed that reactivity did not differ between groups prior to the eyeblink conditioning session ($F(1,84) = 3.18$, $p =$ ns), and was therefore not likely influencing differences observed in CR acquisition.

4. Discussion

Acquisition of defensive learning and avoidance responses are increasingly appreciated as integral to the development of anxiety disorders [8,26–28]. Although avoidance learning has been examined extensively in animal models, often using fear-conditioning paradigms [14,29,30], work in human participants is limited and subject to constraints of US aversiveness. Studies using classical eyeblink conditioning to test avoidance acquisition in human subjects has only been examined across a handful of studies and

without respect to individual differences [19,20,31,32]. Thus until recently [9], the impact of temperament on cue- and response-outcome learning had remained unexplored. In the present study, acquisition in groups receiving omission training did not exceed that apparent during a yoked partial reinforcement schedule of US delivery, suggesting that an avoidance response was not acquired. Temperament, however, was found to modulate learning, with BI individuals demonstrating enhanced CR performance overall relative to non-inhibited individuals. This pattern of enhancement was significantly greater under non-optimal reinforcement conditions.

Degraded acquisition during CR-dependent US omission relative to delay training has been reported in prior eyeblink conditioning studies examining avoidance learning [19,20,31,32], which is consistent with the present study results. Several of these studies, however, also found differences between omission training relative to yoked controls, leading to an interpretation that avoidance responses were acquired [20,21,33]. In the present study, the omission trained and yoked contingency groups demonstrated similar rates of conditioned responding and asymptotic performance, which contradicts findings from previous studies in human participants as well as work in animals reporting avoidance learning [12]. While there were several methodological differences among prior human avoidance studies, such as CS modality employed and use of a 'ready' signal before each trial, another possible source of variation between the results of the present work and that of the prior conditioning studies may be an effect of CS duration.

It is well known that a range of CS–US intervals are sufficient to support CR acquisition in human subjects [34]. A 500-ms CS in the present study was chosen for its effectiveness in eliciting moderate rates of conditioned responding in human participants, enabling both enhancements and decrements in learning to be apparent. It is possible that the use of a slightly longer CS in prior work facilitated acquisition of avoidance learning, making differences between conditioned responding during omission training relative to the yoked comparison groups more perceptible. For example, CR performance of omission groups reached percentages in excess of 70% in some early avoidance studies [20,21], while in the current study asymptotic performance plateaued around 50% and was similar in both omission and yoked contingencies. In one study using a CS duration of 470-ms and a shock US [19], the level of acquisition in the omission-trained group was comparable to that reported herein, although no yoked control group was included for comparison. Taken together, further work testing the influence of varied inter-stimulus-intervals would be necessary to confirm the presence and degree of instrumental avoidance learning in human participants. It would also beneficially inform our understanding of individual sensitivities to control over a predictable aversive outcome.

In the absence of evidence of avoidance learning in the current study, omission and yoked groups did demonstrate the degraded effects on CR acquisition provided by partial reinforcement of the CS relative to participants receiving full (100%) reinforcement. The effect of partial reinforcement on conditioned responding has been well established across species in both classical eyeblink and fear conditioning studies [35,36]. Additionally, it is under the conditions of partial reinforcement (both omission training and its respective yoked control), that learning differences between BI and NI were magnified. When these groups receiving identical schedules of CS/US pairings were combined and evaluated with respect to BI, acquisition was significantly greater in anxiety vulnerable relative to non-vulnerable individuals. This finding is noteworthy, considering that NI individuals received 58% paired CS/US trials on average, while the BI groups received only 49% CS/US pairings. With these reinforcement parameters, conditioned responding in BI might have been expected to result in fewer, or at the very least, equal amounts of CRs relative to NI individuals. However, even when receiving roughly 10% fewer paired trials and a decreasing number of US presentations as the training session progressed, acquisition in inhibited individuals was similar early in training, and continued to increase over the last half of the acquisition session.

Behavioral inhibition has been associated with greater reactivity to negative cues and noxious events and stimuli. Thus it is possible that the observed learning enhancement in BI may have been influenced by sensitivity to the conditioning stimuli. To account for the potential influence of reactivity to the US, which is known to affect rates of learning acquisition [37], UR magnitudes were measured in each participant prior to beginning the conditioning session. Reactivity to the airpuff US was found to be similar between groups with respect to BI and to contingency group assignment, indicating that enhanced CR acquisition was not a result of US sensitivity. Furthermore, there was no correlation between BI and UR magnitudes, $r = .11$, and a relatively large range of inter-individual variability (range: .0.28–6.20, mean: 3.7), suggesting that the lack of difference between BI and NI groups was not likely attributable to ceiling effects of UR magnitudes.

Further potential sources for learning enhancement in BI may stem from a general bias toward to defensive learning acquisition, or a more specific sensitivity to conditions of uncertainty elicited by intermittent CS/US pairings. For example, the use of non-optimal learning contingencies in the present study may have degraded acquisition to a point where learning differences became significant between BI and NI groups, which was evident in pattern but not significance during delay training. Although the interpretation of a general defensive learning enhancement in BI may be sufficient to explain facilitated acquisition reflected in the BI $\times$ Block interaction, it may not fully substantiate the augmentation elicited during the degraded reinforcement contingencies. During the presentation of a non-optimal reinforcement schedule, acquisition in BI and NI individuals was equal early in training and gradually increased over time in BI individuals while responding in NI individuals leveled off by block 2. If BI individuals were sensitive to acquiring a defensive response, they may be expected to demonstrate faster acquisition relative to NI individuals early in training. However, BI groups showed equal rates of CR acquisition early in training, and an increasing number of defensive CRs as the amount of US presentations was being incrementally reduced. This would suggest that factors other than, or in addition to, sensitivity to defensive learning may have been functioning to promote enhanced acquisition in these inhibited individuals.

Alternatively, BI individuals in the present work may have been sensitive to the uncertainty of US reinforcement provided by the partial reinforcement training contingency, resulting in enhanced attention to the conditioning stimuli, or a hypervigilant autonomic state. Such phenotypes are known to support facilitated CR acquisition in eyeblink conditioning paradigms [38]. Indeed, as the session progressed, CS/US pairings became increasingly sporadic, which may have intensified the apparent ambiguity of the CS and US relationship. If BI individuals were more sensitive to this ambiguous situation, diminished influence of the partial reinforcement contingency would occur, resulting in faster acquisition. The current findings of greater generalized responding to the CS observed in anxiety vulnerable individuals has implications for disorder pathology, and in particular, the attribution of an aversive or negative bias to ambiguous cues [7,26].

During simple delay conditioning, acquisition of the eyeblink CR is known to be dependent on the cerebellum [39]. For more complex learning conditions, such as in discrimination/reversal learning, trace conditioning, and partial reinforcement, response acquisition is affected by lesions of extra-cerebellar regions like the hippocampus [40,41], and areas of the medial prefrontal cortex [42]. Although the exact relationship between cerebellar–thalamic–prefrontal connections and complex eyeblink conditioning paradigms is not completely clear, involvement of

the medial prefrontal cortex in modulating acquisition under non-optimal, partial reinforcement conditions has been established [42–44]. With respect to inhibited temperament, enhanced cortical activation and frontal EEG asymmetry has been reported in inhibited infants and toddlers [45], while further work examining neural activation in BI individuals, although limited, has revealed evidence of enhanced activation in limbic regions and in the cerebellum [46]. Thus, within the distributed system of neural substrates involved in CR acquisition, activational differences may be related to learning enhancements apparent in inhibited groups, particularly under non-optimal conditions.

Classical learning is increasingly appreciated as an elemental component of anxiety psychopathology. However, relatively little work has explored associative learning processes and response acquisition in anxiety vulnerable, behaviorally inhibited individuals. Prior work has also shown that expression of avoidance symptoms after trauma determines which individuals will or will not develop a clinical disorder over time [28,47], suggesting that sensitivity to the acquisition and expression of avoidance responses contributes to progression in anxiety vulnerable individuals. In the present work, BI groups demonstrated enhanced response acquisition during the presentation of unpredictable, mildly aversive cues which may be due to a general sensitivity of defensive learning acquisition, or greater vigilance toward ambiguity. While the parameters of the current work were not sufficient to elicit acquisition of an instrumental avoidance response per se, future work examining avoidant behavior would provide greater insight into learning- and avoidance-based disorder etiology in behaviorally inhibited individuals.

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