Behavioural Brain Research 278 (2015) 476–481

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Facilitated acquisition of standard but not long delay classical eyeblink conditioning in behaviorally inhibited adolescents

M.D. Caulfield[a,b], K.M. VanMeenen[b], R.J. Servatius[a,b,*]

[a] Stress and Motivated Behavior Institute, USA
[b] Graduate School of Biomedical Sciences, New Jersey Medical School, Rutgers Biomedical and Health Sciences, Newark, NJ, USA

HIGHLIGHTS

- Inhibited individuals demonstrate facilitated learning at standard delay (500-ms) eyeblink conditioning.
- There are no differences in learning between inhibited and non-inhibited individuals at long delay (1000-ms) conditioning.
- Facilitated learning of standard delay eyeblink conditioning supports a cerebellar role in anxiety vulnerability.
- Adolescents demonstrate similar learning in delay and long delay eyeblink conditioning to young adults.

ARTICLE INFO

Article history:
Received 26 March 2014
Received in revised form 10 October 2014
Accepted 21 October 2014
Available online 30 October 2014

Keywords:
Anxiety vulnerability
Behavioral inhibition
Delay eyeblink conditioning
Long delay eyeblink conditioning
Adolescent anxiety

ABSTRACT

Adolescence is a key age in the development of anxiety disorders. The present study assessed the relationship between behavioral inhibition, a risk factor for anxiety typified by avoidance, and acquisition of the classically conditioned eyeblink response. 168 healthy high school students (mean age 15.7 years, 54% female) were given a battery of self-report measures including the Adult Measure of Behavioural Inhibition (AMBI). The study compared acquisition of three experimental training conditions. Two groups were given paired CS–US training: standard delay of 500-ms or long delay of 1000-ms with CS overlapping and co-terminating with a 50-ms airpuff US. A third group received unpaired training of 1000-ms CS and 50-ms airpuff US. Inhibited individuals showed greater acquisition of the conditioned eyeblink response in the 500-ms CS condition, but not in the paired 1000-ms condition. No differences in spontaneous blinks or reactivity to the stimulus were evident in the 1000-ms unpaired CS condition. Results support a relationship between associative learning and anxiety vulnerability that may be mediated by cerebellar functioning in inhibited individuals.

1. Introduction

Adolescence is a key period for the development of anxiety disorders. With a median age of 11, onset of anxiety occurs much earlier than any other psychiatric illness. Furthermore, half of all lifelong cases of clinical anxiety begin by age 14 [1,2]. The sensitive period of adolescence provides a unique opportunity to study the development of anxiety disorders. So far, it appears that a combination of vulnerabilities contribute to increased risk for developing clinical anxiety. In this vein, a stress-diathesis model emphasizes that the convergence of such factors including genetics, biology, sex, prior experience and personality alters reactivity to stressors in the environment [3]. Recent research suggests that individual differences in learning may also be an important risk factor for anxiety vulnerability [4–6].

Behavioral inhibition (BI) is a personality factor linked to the development of anxiety disorders [7–9]. BI is observable early in life and persists through the lifespan [10]. Individuals with BI demonstrate similar physiological and behavioral profiles as those with clinical anxiety including altered heart rate reactivity [11,12], adrenocortical activity [13], apprehension, withdrawal and avoidance [14,15].

Avoidance is a key symptom of both behavioral inhibition and clinical anxiety [12,16,17], suggesting it is an essential component in the development and maintenance of anxiety. Avoidance is a learned response that is acquired and reinforced over time. As such, avoidance can be measured by assessing acquisition of negative reinforcement contingencies. Those vulnerable to anxiety

* Corresponding author
E-mail address: servatrj@njms.rutgers.edu (R.J. Servatius).

http://dx.doi.org/10.1016/j.bbr.2014.10.027
0166-4328/

disorders may be more susceptible to acquire and repeatedly express avoidant behaviors, leading to the avoidant thoughts and behaviors associated with clinical anxiety.

It is still unclear which factors underlying avoidance acquisition are essential in the development of anxiety. One possibility is that anxious individuals are more sensitive to the cues and contingencies in their environments, resulting in faster learning and better performance on avoidance tasks. This theory is supported by the observation of individual differences in learning in both operant and classical conditioning avoidance paradigms [4–6,18,19]. Although operant paradigms may seem more suited to the high cognitive processes typically associated with avoidance, eyeblink classical conditioning is an established and reliable model for understanding human learning. In classical eyeblink conditioning, a conditioned stimulus (CS) and unconditional stimulus (US) are repeatedly paired, resulting in the acquisition of a conditioned response (CR), the measure of learning. Eyeblink classical conditioning is also one of the few preparations that has an advanced understanding of the neural substrates underlying acquisition with general consensus that the cerebellum is both necessary and sufficient to acquire standard delay eyeblink conditioning [20–23]. Variations of this basic paradigm have shown that rates of acquisition are affected by prior experience with the CS and US (e.g., proactive interference; [4]), by altering the reinforcement schedule [6], or by adjusting the contingencies between the CS and US such as in long delay and trace paradigms [24].

The effects of development on the acquisition of the conditioned eyeblink response have been assessed at length in infants and adults, largely overlooking the period of adolescence. Eyeblink conditioning has been used to demarcate the development of key underlying neural substrates in infants and young children [25–28]. Research in adults concentrates on aging to understand the neurobiology underlying age-related memory disorders [29–32]. Considering that adolescence is a critical period in refining cortical connections as well as for the development of psychopathologies such as anxiety and schizophrenia [33–35] understanding how eyeblink conditioning is affected may shed important light on underlying neural networks.

Using eyeblink conditioning, we found that college-aged participants who score high on the Adult Measure of Behavioural Inhibition (AMBI), a self-report measure of behaviorally inhibited temperament, demonstrated significantly faster learning in a standard delay (500-ms) conditioning paradigm[5]. At face value, this indicates that there is something fundamentally different about how behaviorally inhibited individuals learn about the basic stimuli in their environments, regardless of valence. However, the underlying processes are still unknown. The purpose of the present study was twofold: First, we utilized a basic science approach to assess acquisition of standard delay eyeblink in an adolescent sample for comparisons to other age groups. Second, we addressed two possible theories underlying facilitated learning observed in anxiety vulnerable individuals by comparing acquisition of standard delay (500-ms) to long delay (1000-ms) CS durations. Longer CS durations have slower ontogenetic development [27] and can be more difficult to acquire than standard delay durations [24,27,36]. Additionally, long-delay and trace paradigms demonstrate similar learning curves, with a reduction of learning in long delay, and a drastic reduction in trace paradigms following hippocampal lesion in rats [24,36].

Although delay conditioning is typically considered as cerebellar and trace conditioning as hippocampal, the dichotomy between the two paradigms is not so clear-cut. Evidence suggests the hippocampus is involved during delay eyeblink conditioning. Pyramidal neurons in the hippocampus show increased responding during the CS period in conjunction with development of the behavioral CR, declining with continued training [37]. Neuroimaging studies reflect a similar pattern of activity in the hippocampus during delay eyeblink acquisition [38,39], suggesting that although the hippocampus is not essential in standard delay eyeblink acquisition it still plays a role under normal learning circumstances.

Although the hippocampus is involved during normal learning, hippocampal lesion typically does not affect delay eyeblink conditioning and can actually enhance learning, suggesting the hippocampus may interfere with cerebellar functioning [40]. This is supported by studies assessing acquisition of eyeblink conditioning following hippocampal lesion in delay, long delay, and trace paradigms. Beylin et al. [24] found that unlesioned rats took longer to acquire long delay eyeblink conditioning than standard delay, with acquisition rates similar to that observed in trace conditioning. Additionally, acquisition of long delay eyeblink conditioning was significantly slower in the hippocampal lesioned rats, suggesting that the hippocampus plays a role in its acquisition. Developmental work supports this finding, demonstrating that infant rats can acquire short-delay conditioning, but are impaired at acquiring both long-delay and trace conditioning, which emerges in parallel later in development [41,42]. Therefore, long CS durations provide a useful paradigm to explore the influence of the hippocampus on acquisition without altering the conditioning parameters as drastically as trace conditioning would.

Comparing acquisition of eyeblink conditioning in long and short delays provides a means for understanding whether the neural basis for enhanced acquisition in BI is primarily through reduced hippocampal involvement. Reduced hippocampal involvement in BI would be evident as faster short delay (as previously observed; [5]), concomitant with impaired long delay. If BI is associated with faster acquisition at both short and long delay, this finding suggests that either the facilitation is primarily mediated through the essential cerebellum brainstem/cerebellar circuitry or through those modulatory sites whose influence are in the same direction (accentuation of excitatory influence or diminution of inhibitory influence).

2. Materials and methods

2.1. Participants

168 participants were recruited from a local public high school in New Jersey. Participant's ages ranged from 13 to 19 ($M = 15.7$, $SD = 1.25$). Parental consent forms were signed prior to participation for all students, as well as informed assent (participants under 18) or informed consent (18 and over) in accordance with procedures approved by the high school and University of Medicine and Dentistry of New Jersey Institutional Review Board.

2.2. Self report measures

Participants completed self-report measures including the Adult and Retrospective Measure of Behavioural Inhibition (AMBI/RMBI: [43]), and the State/Trait Anxiety Inventory (STAI; [44]).

The Adult and Retrospective Measure of Behavioural Inhibition (AMBI/RMBI; [43]) is a self-report measure that assesses inhibition or avoidance in response to new stimuli or social situations. It is reliable and has high discriminant validity in separating anxiety, depression, and control groups [43]. Scores on the 16-item AMBI range from 0 to 32 and include questions about current behaviors such as "Do you tend to withdraw and retreat from those around you?", and "Do you tend to introduce yourself to new people?". Scores on the 18-item RMBI range from 0 to 36 and include questions about childhood (during elementary school) behavior.