Neurobiology of Learning and Memory 91 (2009) 415–423

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Learning strategies during fear conditioning

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ARTICLE INFO

Article history:
Received 26 September 2008
Revised 20 December 2008
Accepted 29 January 2009
Available online 7 February 2009

Keywords:
Cortisol
Spatial learning
Classical conditioning
Social aggression
Trout
Fish
Learned escape

ABSTRACT

This paper describes a model of fear learning, in which subjects have an option of behavioral responses to impending social defeat. The model generates two types of learning: social avoidance and classical conditioning, dependent upon (1) escape from or (2) social subordination to an aggressor. We hypothesized that social stress provides the impetus as well as the necessary information to stimulate dichotomous goal-oriented learning. Specialized tanks were constructed to subject rainbow trout to a conditioning paradigm where the conditioned stimulus (CS) is cessation of tank water flow (water off) and the unconditioned stimulus (US) is social aggression from a larger conspecific. Following seven daily CS/US pairings, approximately half of the test fish learned to consistently escape the aggression to a neutral chamber through a small escape hole available only during the interaction. The learning curve for escaping fish was dramatic, with an 1100% improvement in escape time over 7 days. Fish that did not escape exhibited a 400% increase in plasma cortisol and altered brain monoamine response to presentation of the CS alone. Elevated plasma cortisol levels represent classical fear conditioning in non-escaping fish, while a lack of fear conditioning and a decreased latency to escape over the training period in escapers indicates learned escape.

1. Introduction

Fear memories that affect animals living in natural conditions are influenced by contextual information that may be significant for more than just the memory itself. Natural fear learning and conditioning is evoked by stimuli such as territorial competitors and predators that are directly related to how the animal survives. For example, fearful memories of a bigger and stronger territorial competitor include important spatial and social information such as territorial boundaries, food, mates, opponents and rank recognition that add salience to the fear memories of the aggressor. The characteristics of ecologically relevant fear learning and memory formation drove the development of an experimental model that takes specific contextual and social significance into account. Stimuli that evoke social fear are often unpredictable, not habituated, and therefore result in significant stress (Summers et al., 2005). Social stressors have been judged to be the most potent stressors, even for dominant individuals that win aggressive interactions (Koolhaas, de Boer, De Rutter, Meerlo, & Sgoifo, 1997).

Natural and domesticated populations of a wide variety of vertebrates appear to cope with stressful situations, including both social and physical stressors, with a simple dichotomy of heritable strategies; that is, they respond either proactively or reactively (Benus, Bohus, Koolhaas, & van Oortmerssen, 1991; Koolhaas et al., 1999). The proactive phenotype is characterized by behaviorally active coping, such as active avoidance, aggression, or flight responses and low hypothalamus–pituitary–adrenal (HPA) axis responsiveness, but high sympathetic reactivity. The reactive phenotype exhibits passive coping, conservation, immobility, withdrawal, low aggression, elevated HPA responsiveness, and limited sympathetic reactivity. A genetic basis for the expression of behavioral and physiological components of individual coping styles has repeatedly been demonstrated (de Boer, van der Vegt, & Koolhaas, 2003; Driscoll et al., 1998; Ellenbroek & Cools, 2002; Veenema, Meijer, de Kloet, & Koolhaas, 2003).

These divergent characteristics have been artificially selected for in rainbow trout (*Oncorhynchus mykiss*) and exhibit a moderate to high degree of heritability (Pottinger & Carrick, 1999). The two genetically divergent lines differ in their neuroendocrine responsiveness to physical stress (confinement). Trout that respond to confinement stress with highly elevated plasma cortisol levels (high responders, HR) also have a high locomotor response to stress, and do not recover from other stressful events quickly (Øverli, Pottinger, Carrick, Øverli, & Winberg, 2002). In contrast, trout that have a more muted elevation of cortisol response to confinement stress (low responders, LR), have a reduced locomotor response in a territorial intrusion, a more rapid recovery of feeding after transfer to a novel environment, and tend to become socially dominant (Pottinger & Carrick, 2001; Øverli et al., 2002).

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1074-7427/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.nlm.2009.01.009

Stress responsiveness has also been used to demonstrate classical conditioning in rainbow trout (including LR and HR fish) to an aversive event (Moreira, Pulman, & Pottinger, 2004). Pairing an auditory conditioned stimulus (turning the water to the tank off = CS) with an aversive unconditioned stimulus (confinement stress = US) over time produces a conditioned response of elevated plasma cortisol concentrations to the CS alone. Strain differences in the ability to form or recall memories of a stressful event after a similar number of training trials are demonstrated by a more rapid rate of extinction of the conditioned response in HR fish (Moreira et al., 2004). This kind of conditioning is suggestive of classical fear conditioning in rodents that pairs electric foot shocks (US) with auditory or visual stimuli (CS) to produce behaviorally conditioned responses such as freezing or potentiated startle (Davis, 1980), but replaces the behavioral effect with a physiological one. This conditioned physiological response suggests that stress coping styles may be an evolutionary adaptation that includes learning (Øverli et al., 2007). In addition, physiological responses to fear conditioning in a natural setting may influence behaviorally relevant outcomes to aversive stimuli and contextual spatial elements of the environment in which they occur.

We propose a model for fear conditioning using rainbow trout that combines the aversive stimulus of a larger territorial competitor as an unconditioned stimulus that promotes fearful behavioral and physiological responses, while providing an opportunity for a smaller test fish to learn to escape from an aggressive interaction that it cannot win. We hypothesized that over a 7 day trial, a majority of the resident trout will learn to escape from an aggressive interaction into the safety of an adjacent chamber. Furthermore, we hypothesized that the escape behavior can be a product of a conditioned response to a neutral stimulus.

Our results suggest two divergent types of learning. In response to the presence of a much larger aggressive conspecific, test fish display two distinctive behavioral responses, escaping or remaining. Learned escape is characterized by a rapid decrease in the latency to escape over seven training periods (see Fig. 1), but notably, an absence of escaping as a conditioned response. Those fish that do not escape, and remain with the aggressive US each of the 7 training days display (see Fig. 1) classical fear conditioning to presentation of the neutral CS alone. The conditioned response is manifest by physiological and neurochemical responses.

2. Materials and methods

All work with fish was conducted at the Gavins Point National Fish Hatchery in Yankton, South Dakota. Prior to experimentation, Rainbow trout *(O. mykiss;* raised from eggs) in size matched groups were housed indoors in six foot diameter circular fiberglass tanks under natural light conditions. Fish were fed daily with Nelson's Silver Cup trout feed at a rate of 1% body weight per day. These experiments were conducted in a manner that minimized suffering of subjects as well as the total number of animals used in accordance with the Declaration of Helsinki and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23), under approved protocol by the University of South Dakota IACUC.

2.1. Tanks

Test aquaria were 75 gallon flow through systems, individually lit, with water inflow spray bars on both sides. Each tank was divided into three separate chambers, with space for a test fish in the middle compartment, a large fish on one side and an empty chamber on the other side (Fig. 1). Chambers were formed by the insertion of opaque barriers which could be easily removed and served to elicit territorial association with a specific space, and to eliminate contact between fish. Two barriers were inserted between the test fish chamber and the empty chamber, such that when one barrier was removed, a small escape hole (2 in. diameter) became available for use during the interaction (the escape hole remained covered at all other times). This escape hole was large enough for the test fish, but not the larger fish to pass through. Importantly, before experimental day one, test fish had no exposure to the escape hole.

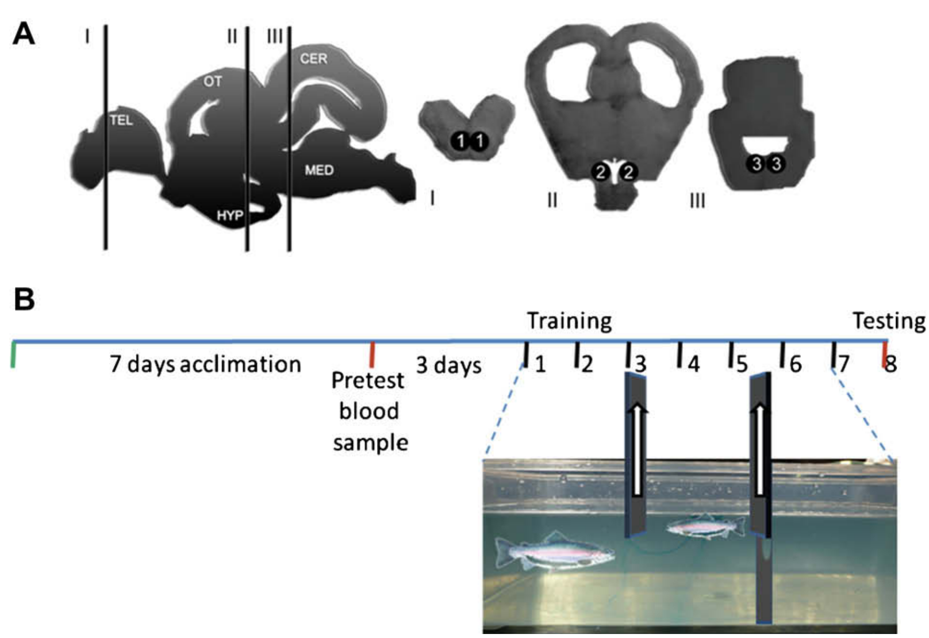

Fig. 1. (A) Anatomical representation for specific regions chosen for microdissection. Sagittal section of a rainbow trout brain; TEL = telencephalon, OT = optic tectum, HYP = hypothalamus, CER = cerebellum, MED = medulla. I Telencephalic coronal section ❶ represents striato–amygdalar complex (subpallium). II Diencephalic coronal section ❷ hypothalamic region sampled. III Brainstem coronal section ❸ raphé. (B) Time line of experimental design. Days 1–7 fish were acclimated to the experimental tanks. A pretest blood sample was taken following acclimation, and fish were allowed to recover for 3 days. Training (CS + US) occurred daily over the next week, with testing on the following day. Fifteen minutes after the initiation of testing, terminal blood and brain samples were collected for analysis.