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BRIEF COMMUNICATION

Overtraining Reduces Morphine Abolition of Classically Conditioned Responses

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MAUK, M. D., T. G. CASTELLANO, J. A. RIDEOUT, J. MADDEN, IV, J. D. BARCHAS AND R. F. THOMPSON. *Overtraining reduces morphine abolition of classically conditioned responses.* PHYSIOL BEHAV **30**(3) 493–495, 1983.—We have previously reported that intravenous injection of morphine produces selective abolition of recently learned, aversively motivated classically conditioned responses. We now report that morphine administered to overtrained animals produces considerably less effect on conditioned responding than in animals recently trained. This effect cannot be accounted for by factors related to consolidation. Results are discussed in relation to a two process model of aversive learning.

Classical conditioning Opiates Conditioned fear Overtraining Two-process model Cerebellum

WE have previously reported a series of studies demonstrating that administration of opiates in recently trained rabbits produces selective abolition of simple, aversively motivated conditioned responses: the classically conditioned nictitating membrane (NM) and eyelid responses [12–14]. This effect is mediated by activation of opiate receptors within the central nervous system [12,13]. Performance of the reflex unconditioned response (UR) and conditioned stimulus-evoked neuronal discharges in the primary auditory pathway are both unaffected. Thus, opiates are acting centrally to produce a selective, and perhaps a direct effect on some portion of neural circuitry essential for the learned response.

There have been suggestions in previous literature that the effects of morphine on conditioned responses fade as a result of overtraining [3,9]. In our previous expeirments, animals were trained to a high level of conditioned responding, but were not overtrained. This report describes the effect of overtraining on the ability of intravenously administered morphine to abolish conditioned responses. The results are discussed within the context of a two process model of aversive learning.

METHOD

A total of 32 male, albino rabbits (*Oryctolagus cuniculus*) were used. Each rabbit was surgically prepared under halothane anesthesia with a headstage designed to accommodate the stimulus delivery/micropotentiometer assembly. Animals were allowed at least five days of recovery before training began.

Training and testing procedures have been described elsewhere in detail [27]. Briefly, training consisted of short delay classical conditioning with an auditory conditioned stimulus (CS; 1 kHz, 85 dB, 350 msec) and a corneal airpuff unconditioned stimulus (US; 2.1 N/cm^2 pressure, 100 msec, coterminate with the CS). Training blocks were comprised of eight CS-US paired trials and one CS-alone test trial. Extension of the nictitating membrane was transduced by micropotentiometer and recorded on polygraph (Grass model 7 D). A CR was defined as a NM extension of at least 0.5 mm within the CS-US interval. Animals were trained to a criterion of eight CRs in any nine consecutive trials and then given varying amounts of overtraining blocks before being administered morphine (5 mg/kg IV). CR peak amplitude was determined for each trial in the block before, and the three blocks after, morphine injection. Data were then converted to percent of the pre-injection baseline.

One group of animals was given the same amount of pre-injection training as reported in previous experiments (two blocks beyond criterion, n=15). Two other groups were overtrained nine blocks (n=4), or 15–26 blocks (n=7). Overtraining the animals 15–26 blocks required one to two additional training sessions, allowing for potential confounding by consolidation factors (i.e., time-dependent changes, see [18]). Accordingly, an additional group (n=6) was trained to criterion and removed immediately. One to two days later,

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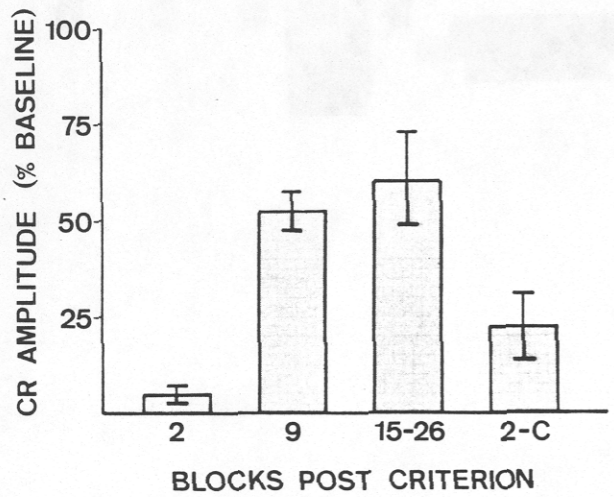

FIG. 1. Conditioned response amplitude following a 5 mg/kg injection of morphine expressed in terms of percent of pre-injection baseline. Each group received different amounts of overtraining before injection: 2 blocks (n=13), 9 blocks (n=4), 15-26 blocks (n=7). The final group (2-C) was the consolidation control (n=6).

matched with animals in the 15–26 group, the animals were given two blocks of training and then administered morphine. In previous studies (12–14) control injections of saline or Ringer's solution vehicle had no effect (i.e., 100% on Fig. 1).

Morphine sulfate injections, 5 mg/kg body weight (see [14]), were administered via the lateral ear vein with 25 gauge catheters. Injection volumes were 0.25 ml/kg or 0.5 to 0.75 ml per rabbit.

RESULTS

As Fig. 1 indicates, overtraining greatly reduces the effect of morphine on conditioned responses. The effect is statistically reliable, $F(3,28)=14.80$, $p<0.001$. Post hoc Newman-Keuls analysis revealed that the animals overtrained nine blocks and those overtrained 15–26 blocks were reliably different from the animals trained only two blocks past criterion ($p<0.01$). Although the consolidation group showed a reduced effect numerically, the comparison with the 2-block group was not statistically reliable. The consolidation group was, however, reliably different from both the 9-block and 15–26 block overtraining groups ($p<0.05$ and $p<0.01$, respectively). Thus, this effect of overtraining on opiate abolition of CRs cannot be accounted for by factors related to consolidation.

DISCUSSION

We have demonstrated in this experiment that overtraining markedly reduces the vulnerability of conditioned responses to challenge by intravenous injection of morphine. This reduced effect appears to be related to the presentation of additional training trials rather than to consolidation per se.

Theoretical accounts of aversively motivated learning have emphasized two processes: a rapidly acquired conditioned central state (e.g., conditioned drive state or conditioned fear) and the subsequent development of discrete, striated muscle responses adaptive to the situation [8, 21, 22, 24]. We have proposed, in the spirit of Konorski [8], that the conditioned central state is necessary for the development of discrete, adaptive responses and that opiate abolition of conditioned NM responses early in training reflects an action on conditioned drive states or conditioned fear [26,28]. In general support of this notion, administration of opiates produces selective abolition of CRs in a wide variety of situations in which conditioned fear modulates behavior (conditioned heart rate responses, [4,10]; conditioned emotional response, see [20]; escape-avoidance conditioning, see [6]; startle response potentiation, [2]).

Several lines of evidence suggest that the conditioned central state or conditioned fear is not invariant throughout the course of training. The autonomic and "non-specific" responses generally considered indices of conditioned fear [7] develop rapidly, usually in 5–15 trials, well before specific, adaptive responses appear (see [29]). Several reports have demonstrated that these autonomic CRs fade at about the time conditioned striated muscle responses develop [23, 25, 30]. In a more direct test, Mineka [19] found that as discrete, adaptive responses become stronger (more resistant to extinction) the conditioned emotional response to the same CS declines. Thus, the present data are not necessarily inconsistent with the notion that opiate abolition of NM CRs is a result of action on conditioned fear. In fact, the present data appear to be consistent with the apparent dissociation between conditioned central states and adaptive, striated muscle responses that develops throughout the course of training.

One possibility is that the neural structures subserving the discrete adaptive responses develop a certain degree of functional autonomy with overtraining. We have recently presented considerable evidence indicating that the neocerebellum is essential for the learning of CRs involving discrete, striated muscle responses [1, 11, 15–17]. Ipsilateral cerebellar lesions completely block the development of conditioned NM/eyelid responses and produce complete and selective abolition of previously learned NM/eyelid CRs. Unlike the effects with opiates, this lesion effect occurs even in overtrained animals. These lesions do not appear to affect the development of conditioned fear. Animals with bilateral cerebellar lesions show no impairment in the development of heart rate CRs, even though they are unable to learn NM/eyelid responses (unpublished observations). Removal of the cerebellum also has no effect on the development of heart rate CRs in the pigeon [5].

Other interpretations are possible. The reduced opiate effect reported here may simply reflect a single neural substrate of the conditioned response that becomes increasingly resistant to opiate challenge. Regardless, some portion of the neuronal circuit essential for the expression of the learned response is affected by opiates early in training yet is relatively unaffected with extended training. Thus, characterization of the precise mechanism of opiate action may identify certain portions of the neural circuit essential for the learned response as well as how it changes throughout training.

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