CORRESPONDENCE

Anesthesiology
82:308–309, 1995

What Is a "Replication"? Epinephrine Facilitation of Learning under Anesthesia

To the Editor:—Replication is the *sine qua non* of science. Therefore, a reported failure to replicate necessarily raises doubts about the validity of the original findings. Recently, El-Zahaby *et al.* reported in ANESTHESIOLOGY[1] a failure to replicate the findings of Weinberger *et al.*[2] that epinephrine facilitates learning under anesthesia. El-Zahaby *et al.* concluded as follows:

> Two reports in the literature have influenced the recent surge of interest in learning during anesthesia and have been cited often. One of them is Weinberger *et al.*'s work in animals, and the other is Levinson's [citation given] study in humans. . . . It is therefore *disturbing* [italics added] that we could not replicate the essential aspects of one study [referring to Weinberger *et al.*] and another group could not replicate the other [citation given].

Two other points are cited to cast doubt on the Weinberger *et al.* findings. The first questions the validity of the conditioned suppression test that we used to assess learning 10 days after training. The second is their statement that there have been no prior replications of Weinberger *et al.*

Readers may thus conclude that the Weinberger *et al.* findings were not genuine. However, none of these points are valid. First, the failure of El-Zahaby *et al.* to replicate is based on their performing an experiment that differs in so many major respects from Weinberger *et al.* that it constitutes an attempt to *extend* the findings to a different situation rather than an attempt to *replicate*. Second, conditioned fear is known to last for more than 10 days. Third, Weinberger *et al.* have been replicated using the same paradigm and procedures.

A Comparison of the Experiments. The El-Zahaby *et al.* study differed in several major respects from the Weinberger *et al.* study; species (actually Mammalian order) of the subjects, type of anesthetic, depth of anesthesia, training protocol, behavioral response measured, behavioral testing conditions, and behavioral retention interval. Weinberger *et al.* studied rats anesthetized with sodium pentobarbital and chloryl hydrate, trained briefly in a single session, and tested for classical fear conditioning by using conditioned suppression of operant behavior 10 days after training. El-Zahaby *et al.* studied rabbits that were in a subanesthetic state induced by isoflurane, trained extensively in several sessions, and tested for classic conditioning of the nictitating membrane response during acquisition and 2 days later during extinction.

Several of these differences were noted by El-Zahaby *et al.*, and they discussed one, the possibility that the nictitating membrane response is less sensitive than conditioned suppression as an assessment of learning. Given the vast differences between the studies, it is impossible to determine which of the variables is (are) critical. However, it is conceivable that in this situation the nictitating membrane response is less sensitive because subjects learn at least two things: (1) that the conditioned stimulus precedes the unconditioned stimulus (fear conditioning, involving behaviors incompatible with ongoing water licking) followed by (2) learning to make a precisely timed somatic motor response (resulting in the nictitating membrane conditioned response). Fear conditioning, as indexed by conditioned autonomic responses or conditioned suppression, is acquired more rapidly than is the nictitating membrane conditioned response.[3–5]

One major variable was the same for El-Zahaby *et al.* and Weinberger *et al.*, the doses of epinephrine. Paradoxically, the use of the same doses might help explain the different findings. Weinberger *et al.* selected these doses based on prior studies in the waking rat that had shown facilitation of fear conditioning and other types of learning. However, apparently there are no published reports of epinephrine facilitation of nictitating membrane conditioning in the rabbit. Thus, the selection of doses by El-Zahaby *et al.* appear to be based on the rat and on a different aspect of learning. Therefore, one of the many possible reasons for the lack of robust facilitation observed by El-Zahaby *et al.* is that their doses may not have been optimal for the rabbit in their training situation. It might be helpful to first establish the appropriate facilitating doses for the nictitating membrane conditioned response in the normal rabbit to provide dose-response functions that could be used to guide the study of learning and anesthesia.

Interestingly, El-Zahaby *et al.* did report a statistically significant facilitation of the 0.01-mg/kg dose of epinephrine on day 6 of acquisition training. However, no effects were found in subsequent extinction training. Of note, the group means of the facilitating dose were greater than for the control and other epinephrine group also on days 4 and 5 (see their fig. 5). These findings suggest that the authors may have obtained a weak effect that might be made stronger if intragroup variability could be reduced, if other doses of epinephrine are used, or both.

Other Attempts to Replicate Weinberger *et al.* The second point is that there have been no previous replications of Weinberger *et al.* that used the same procedures. That is incorrect. In 1985, Gold *et al.*[6] both replicated and extended the original study by Weinberger *et al.* That this replication was performed by the same authors as in the original study should not be sufficient reason to discount these findings. More recently, another laboratory has reported a replication of Weinberger *et al.*, also using rats and lick suppression.[7]

Retention of Conditioned Suppression. El-Zahaby *et al.* state that there is a lack of evidence that conditioned suppression can be observed as long as 10 days after training. However, fear conditioning is well known to show behavioral evidence of learning and retention in the rat for far longer than 10 days, whether it is assayed by conditioned suppression or by other means. Examples are 25 days (Goldstein[8]), 28 days (Campeau *et al.*[9]), 30 days (Franchina[10]), 35 days (Hendersen[11]), 42 days (Coulter *et al.*[12]), 45 days (Neuenschwander-El Massioui *et al.*[13]), 60 days (Goldstein[14]), and 90 days (Gleitman and Holmes[15]).

Replication *versus* Extension. The El-Zahaby *et al.* paper raises the question of what is meant by a "replication." If this term is to be very helpful to readers, then it should be restricted to circumstances in which either the same experiment is repeated with no more than minor variations or a highly similar experiment is undertaken. Of course, no fixed formula can be applied to the term "highly similar," so that the decision as to whether a study is an attempted replication is likely to remain somewhat subjective unless identical methods are employed. Nonetheless, readers would be better served if authors and editors use a term such as "extension" rather than "replication" whenever the two experiments in question differ greatly. In the present case, it would be clear to readers that El-

Zahaby *et al.* failed to *extend* epinephrine facilitation of learning under anesthesia to a situation of differences in species of subjects, type of anesthetic, depth of anesthesia, type of training, and nature of the behavioral assay of learning. Moreover, authors would be alerted to the distinction between an attempted replication and an attempted extension and thus be less likely to be concerned by failures to replicate that are more apparent than real. The result would be to reduce or preferably avoid confusion and obviate the need for communications such as this letter. The focus then could be on understanding the phenomenon of learning under anesthesia.

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(Accepted for publication October 3, 1994.)

Anesthesiology
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In Reply:—Thank you for giving us the opportunity to respond to Weinberger and Gold's letter. We apologize for not citing the article by Gold *et al.*[1] and for underestimating the durability of conditioned fear. When we stated[2] that ". . . we could not replicate *the essential aspects* [italics added] of one study [referring to Weinberger *et al.*[3]]," it was apparent that, although both groups used classic conditioning paradigms, our results were different, and we could not replicate learning and memory during anesthesia. Weinberger and Gold suggest that we should have used the term "extension" rather than "replication." We have no objection, if this leads to better clarity for the reader. Weinberger and Gold expand on the differences between the two studies, which we have cited in our paper, but these differences cannot account for, in our opinion, the startling differences in the results, *i.e.,* epinephrine enabling learning in anesthetized subjects but failing to do so in subjects receiving subanesthetic doses. Rabbits are more resistant than rats to the effects of anesthetics; enhancement of learning and memory by epinephrine should be more apparent with subanesthetic rather than anesthetizing doses, and a shorter retention interval should favor a more durable memory.[1] Even if fear conditioning is acquired more rapidly than the nictitating membrane conditioned response, our use of six training sessions and 360 training trials *versus* 1 and 10, respectively, by Weinberger *et al.* should mitigate any contribution of the different behavioral assays of conditioning in the two studies to the differing results. Weinberger and Gold suggest that the doses of epinephrine used may explain our different findings. This is unlikely. A look at figure 5[2] shows that we obtained the same *pattern* of enabling effects of epinephrine doses as Weinberger *et al., i.e.,* 0.01 mg/kg epinephrine producing a better effect on learning than 0.1 mg/kg. Therefore, the use of larger doses could not have improved our results. We also had limited preliminary