decision relevant to that behavior that the animal makes must involve the process representing those relations. If, after differential training, an animal is presented with an unfamiliar cue and performs a response appropriate for a cue with which it is familiar, one might assume that it has "remembered" the familiar cue and its associated response: the readout process representing that association has been elicited. One might also assume that there would be instances when the cue and its associated response would be "remembered," yet the response would not actually be performed. Contrary to Bindra's assumption, this is exactly what we have found. I pointed out in my article that the readout process is a necessary but not sufficient correlate of the actual instrumental response.

I agree with Bindra that an inhibitory stimulus, CS—, requiring response suppression, should produce some characteristic readout waveshape, analogous to that produced by a positive stimulus, CS+, albeit different in form. Bindra errs, however, in assuming that this is not the case, although I was at fault for not explicitly stating this fact (*2*). Apparently he was misled by my figures 6 and 7, comparing waveshapes obtained to the same stimulus in go and no-go outcomes. Further examination of the data presented in figures 8, 9, 10, and 11, as well as a careful reading of the text, ought to clarify this point (*1*).

Bindra suggests that the differences between readout waveshapes which we reported primarily reflect motivational processes. It was precisely because of the obvious motivational differences between go and no-go contingencies that we trained some animals in approach-approach (CR_1 and CR_2) discriminations and some in avoidance-avoidance (CAR_1 and CAR_2) discriminations. Clear differences between readout waveshapes elicited by the same stimulus during trials resulting in performance of CR_1 compared with trials resulting in CR_2, and in CAR_1 compared with CAR_2, are documented in figures 10 and 11 and for cats 9, 16, and 21 in table 1 of my article (*1*).

Bindra suggests that a decisive test of his alternative interpretation, that the difference in waveforms reflects a motivational process, could be accomplished by determining whether variations in motivational level produced systematic changes in the amplitude of the readout waveform to CS+. In control studies conducted previously but not reported in my article, the waveshapes elicited by an appetitive CS in hungry and satiated cats were compared. In the satiated condition, not only was the cat fed to satiety but a dish of food was placed beside the response lever on the work panel in the apparatus. These studies showed that neither readout processes associated with the approach CS nor those associated with the avoidance CS were altered by satiation (see Fig. 1). Numerous other control experiments (*1*, *3*) for unspecific processes including motivation support the interpretation that the phenomena defined as the readout process do in fact reflect mnemonic processes. These further experiments show that processes related to a variety of unspecific features, probably including motivational processes, can indeed influence the evoked potential waveshape. Differences in the stimuli themselves also are reflected in the waveshapes. These influences, undoubtedly of intrinsic interest, have explicitly been excluded from contribution to the readout process as we have identified it.

Bindra, in his last contention that memories are instantaneously activated by appropriate cues whereas motivational processes are slow, fails to consider an essential point. The cats in our experiments are put in decision-making situations that are difficult and important. The decision requires identification of the stimulus repetition rate, that is, the length of the time interval *between* identical flashes or clicks. Obviously, it would be impossible for the appropriate memory to be instantaneously activated under these conditions. The time intervals must be sampled enough for the animal to estimate their length, *before* the memory appropriate to a particular repetition rate can be activated. In differential generalization, when the stimulus repetition rate is itself novel, the sample of intervals required for this time estimation must be even larger. Response latencies are prolonged and the animals appear stressed.

Finally, although I believe that I have adequately answered Bindra's questions, I find it necessary to challenge a basic assumption in his comments. I fail to understand how one can separate "motivational" processes from mnemonic ones. Motivational processes reflect the valence attached to an event, and such valences are not intrinsic to a CS but derive from experience. Were the readout processes which we have discovered to reflect the valence attached to a stimulus because of past experience, rather than the broader implications of meaning which I believe to be the case, readout would nonetheless reflect the activation of memory.

E. R. JOHN

Brain Research Laboratories,
Department of Psychiatry,
New York Medical College,
New York 10029

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1. E. R. John, *Science* **177**, 850 (1972).
2. Studies (E. Grastyan, E. R. John, F. Bartlett, in preparation) confirm that in cats trained to a CS+ and a CS—, the waveshape elicited by the CS— does acquire a readout component as an inhibitory response is established.
3. E. R. John, F. Bartlett, M. Shimokochi, D. Kleinman, *J. Neurophysiol.*, in press.
4. This work was supported by NIH grant NS09924, NSF grant GB-27559, and the Health Research Council of New York grant I-752.

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Role of the Skin in Amphibian Sodium Metabolism

McAfee's (*1*) rebuttal of a statement by Kalckar (*2*), and the latter's concurring reply, raises and helps clarify some interesting questions about the role of the skin, and its active Na^+ transport mechanism, in maintaining the sodium balance of amphibians. McAfee's (*1*) measurements of a frog's (*Rana pipiens*) ability to survive, and to maintain body Na^+, when bathed in deionized water helps to resolve the problem of the relative importance of the skin in amphibian osmoregulation. I have been seeking an answer to the same problem but using a different procedure. Instead of preventing Na^+ uptake by excluding it from the bathing media, I have attempted to bring about the same effect by inhibiting its active uptake by soaking amphibians in dilute solutions of amiloride. The conclusions on three species of anurans are in general accord with those of McAfee.

Amiloride (*3*) is a diuretic drug which is a potent (but its action is reversible) inhibitor of active transmural Na^+ influx, although it does not change Na^+ outflux, across several amphibian epithelial membranes. These include

Table 1. Serum Na^+ concentrations (mean ± standard error) of amphibians after bathing for 15 days in $10^{-5}M$ amiloride solution (in tap water). Controls are in tap water (Na^+ 0.25 meq/liter alone.

Skin	N	Serum Na^+		P
		Controls (meq/liter)	Amiloride (meq/liter)	
B. marinus	7	101 ± 2.1	92 ± 2.9	< .05
R. pipiens	9	100 ± 2.0	96 ± 1.1	> .1
X. laevis	8	106 ± 1.4	105 ± 1.5	> .6

Table 2. The potential difference (PD) and short-circuit current (SCC) (mean ± standard error) across amphibian skin (in vitro) after bathing in $10^{-5}M$ amiloride for 15 days. The amiloride was washed from the skins prior to the measurement. For differences in SSC $P < .01$ for *B. marinus*, others $P > .4$.

Skin	N	Controls		Amiloride	
		PD (mv)	SCC (μa/3.8 cm^2)	PD (mv)	SCC (μa/3.8 cm^2)
B. marinus	7	24 ± 2.4	100 ± 12.4	37 ± 3.9	160 ± 4.1
R. pipiens	9	23 ± 3.4	73 ± 6.9	36 ± 6.1	89 ± 19.7
X. laevis	8	25 ± 5.6	24 ± 4.9	39 ± 3.0	25 ± 2.8

the skin of frogs and toads where it acts, only, at the outside (epidermis) surface in concentrations as low as $10^{-7}M$. It is stable in solution and is not significantly metabolized in the body. Frogs normally do not drink (*4*), and, as their skin is impermeable to amiloride (*5*), it would be expected that its action is confined to the outer cutaneous surface. Apart from seeking the answer to the problem in a different manner, I have used amiloride because it is of additional interest as the amphibians continue to be bathed in media containing Na^+. Thus possible interactions due to the presence of a Na^+-free solution (as discussed by McAfee) are excluded.

For my experiments I chose three species of anuran amphibians from different natural habitats: *Xenopus laevis* (which is aquatic), *Rana pipiens* (which is amphibious), and *Bufo marinus* (which is mainly terrestrial). Groups of these fasting animals were soaked for 15 days in tap water (Na content was about 0.25 meq/liter) containing amiloride ($10^{+5}M$). The bathing solution was changed daily. A control group of each species was treated similarly, but bathed in tap water alone. There were no fatalities, and all the animals appeared to remain in good condition. The concentration of amiloride used is adequate, when tested in vitro, to abolish the potential difference (PD) and short-circuit current (SCC), reflecting active Na^+ transport, in 2 to 3 minutes. Experiments in vivo confirm that it also inhibits the net Na^+ uptake (*6*).

At the end of the 15 days the serum Na^+ concentrations of the amphibians (mean ± standard error) are shown in Table 1. The effects, on serum Na^+, of inhibiting active Na^+ uptake were either very small or nonexistent.

As indicated by McAfee, deprivation of external Na^+ may result in compensatory changes which are reflected in the PD and SCC across the skin in vitro. In my experiments these values were recorded (*7*) after the skin was washed free of amiloride (Table 2). In addition, urinary bladders (in vitro) of *B. marinus* showed changes in SCC which paralleled the differences seen in the skin. No such differences were seen in *R. pipiens*. The elevation in SCC observed in the skin (and urinary bladder) of *B. marinus* soaked in amiloride solutions is consistent with a depletion of Na suggested by the decline in the Na^+ in their serums. The lack of such differences in the other two species are similarly compatible with the equality of Na^+ in their serums. Thus over a 15-day period, inhibition of transcutaneous Na^+ transport did not provide any evidence suggesting Na depletion in *R. pipiens* or *X. laevis*, although a modest decline may have occurred in *B. marinus*. The role of the skin in maintaining body Na^+ could differ in various species. However, there is no indication that its role is of acute importance, although over a period of several weeks in water it could play a significant role, especially in fasting animals.

It must be reiterated that the role of the amphibian skin in amphibian Na^+ metabolism is still not clear. As far as I can recall most vertebrate epithelial membranes exhibit transmural Na^+ transport, a property commonly attributed to the need for some process of Na^+ conservation. In some membranes transport of amino acids and sugars is linked to this process (*8*). The skin (and other epithelial membranes) performs other functions, apart from active Na^+ transport, and this includes, in amphibians, a role in rehydration and respiration. It is thus conceivable that transmural Na^+ transport may also play an indirect role in helping to maintain the skin's physiological, metabolic, and even anatomical integrity. We should thank McAfee for raising his interesting question, the answer to which may be of importance to an understanding of epithelial membrane function.

P. J. BENTLEY

Departments of Ophthalmology and Pharmacology, Mount Sinai School of Medicine of the City University of New York 10029

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