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11. Sperm contain another enzyme with diaphorase activity which may be visualized on acrylamide gels under special conditions. There is a membrane bound diaphorase that is extracted from sperm by treatment with 1 percent Triton X-100 but is not removed by the freeze-thaw treatment in water; it migrates close to the origin under the electrophoretic conditions described in the legend to Fig. 1.
12. This report is contribution 185 of the Forensic Science Group, University of California, Berkeley. Supported by an Institutional Biomedical Research Support grant from NIH and by grants 74NI-99-0041 and 74NI-99-1007 from the National Institute of Law Enforcement and Criminal Justice, Law Enforcement Assistance Administration, Department of Justice. We thank many colleagues, particularly Drs. A. C. Wilson, R. P. Erickson, and R. Palmour, for advice and comments. Inquiries regarding our work should be directed to G.F.S.

1 October 1975; revised 5 December 1975

Lateral Preoptic Lesions in Rats Separate Urge to Drink from Amount of Water Drunk

Abstract. *Rats with bilaterally symmetrical lesions in the lateral preoptic area do not drink after acute intracellular dehydration, but they drink normally after water deprivation. They, like normal rats, also drink more when cellular dehydration is superimposed upon water deprivation. Unlike normal rats, however, rats with lesions in the lateral preoptic area do not increase their rate of lever-pressing in response to the combined stimulus. Thus, the urge to drink can be separated from the amount of fluid drunk.*

Dehydrating either the cellular (*1*) or the extracellular (*2*) body fluid phases induces drinking. Drinking in response to cellular dehydration terminates upon restoration of the cellular fluid deficit (*1*). Conversely, water intake following extracellular dehydration stops before the deficit is restored (*2*). This is thought to reflect inhibition by cellular overhydration, which results because approximately 70 percent of the ingested water gains access to the cellular phase (*3*). When cellular and extracellular phases are depleted simultaneously, the resulting water intake is a simple addition of the amounts drunk in response to each challenge when presented alone (*4*). Although additivity of drinking is a robust phenomenon, the underlying mechanisms are not known. Understanding this phenomenon is vital for appreciating how behavior contributes to maintenance of normal fluid balance. This is especially important when we consider that, under normal circumstances of fluid abstinence, water is lost from both fluid compartments.

At least three alternative hypotheses can account for drinking additivity. (i) Additivity may reflect an augmented urge to drink so that more water is needed to quench this enhanced thirst. (ii) By virtue of further dehydration of the cellular phase, an inhibitory signal arising from cellular overhydration may be delayed. (iii) There may be a tonic inhibition exerted by the nondehydrated cellular phase over thirst signals arising from extracellular depletion, and dehydrating the cellular phase may relieve this inhibition.

These alternatives cannot be assessed in normal rats which drink in response to both cellular and extracellular dehydration and reduce drinking because of cellular overhydration before restoration of the extracellular phase. Rats with bilaterally symmetrical lesions of the lateral preoptic area (LPO), which do not drink in response to cellular dehydration but are indistinguishable from normal rats in their drinking response to hypovolemia (*5*), are perfectly suited to test these alternatives. We report that the amount drunk by rats with LPO lesions after water deprivation and after water deprivation combined with cellular dehydration is the same as in normal rats, but the rate at which these brain-damaged rats work for water, unlike that of normal rats, is not affected by superimposing cellular dehydration upon a preexisting water deprivation deficit.

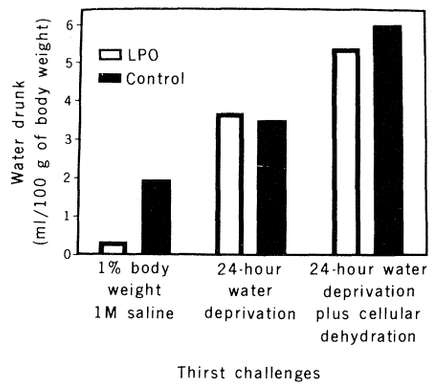

Fig. 1. Amount drunk in 5 hours by rats with LPO lesions and controls after the different thirst challenges.

Ten adult female Sprague-Dawley rats were rendered unresponsive to cellular dehydration by placing bilaterally symmetrical lesions in the LPO (*6*). These rats and an equal number of controls were allowed to drink for 5 hours after acute cellular dehydration (intraperitoneal injections of 1*M* NaCl, 1 percent of body weight), 24 hours of water deprivation, or the combined challenge. As seen in Fig. 1, rats with LPO lesions, which drank nothing or very little in response to the purely cellular stimulus, drank normally after water deprivation and, most importantly, drank as much as normal rats in response to the combined challenge (*7*). These data are not compatible with a theory that volume drunk reflects a simple addition of individual thirsts. However, the results are predicted by an inhibition model, and they suggest that removal or delay of an inhibitory cellular overhydration signal is an adequate explanation for drinking additivity.

Can this reasoning also account for the increased rate of operant responding seen in normal rats with increased dehydration? If increased lever-pressing reflects either a delay of inhibition, resulting from cellular overhydration, or a removal of a tonic inhibition exerted by the cellular phase, then the operant rates of rats with LPO lesions should increase when a cellular dehydration challenge is superimposed upon water deprivation. On the other hand, if the urge to drink mirrors the detection of dehydration of each fluid compartment, then the combined challenge should produce increased response rates in normal rats, which can detect cellular dehydration, but not in rats with LPO lesions, which cannot detect deficits in cellular balance.

Rats with lesions and controls were gradually trained to lever-press on a variable-interval, 30-second schedule of reinforcement when deprived of water for 24 hours. The operant task was specifically designed to reflect changes in the urge to drink independent of satiety and level of intake. Sessions were thus short (30 minutes) and reinforcements were small (0.04 ml); maximum intake per session was lim-

Table 1. Mean number of bar presses by rats with LPO lesions and normals in the 30-minute test session following the various challenges; N.S., not significant.

Rats	Mean responses per session		Change (%)	t_{dep}	P
	Before cellular dehydration	After cellular dehydration			
		No water deprivation			
Normal	11.9	44.1	270	2.48	< .05
LPO	104.0	108.5	4.3	0.55	N.S.
		24 hours of water deprivation			
Normal	106.8	172.9	61.9	6.06	< .001
LPO	234.5	244.6	4.3	1.05	N.S.

ited to less than 2.5 ml. The operant sensitivity was determined by testing the rats after 0, 9, 24, and 48 hours of deprivation, counterbalanced across rats. Deprivation significantly affected operant rates in both groups [rats with LPO lesions: $F = 10.20$, $P < .001$; controls: $F = 15.33$, $P < .001$; one-way repeated measures analysis of variance (*8*, pp. 167–186)].

Surprisingly rats with LPO lesions had a considerably higher operant rate than controls when not deprived. This may reflect a general increase in arousal and activity in these animals (*9*). It is of particular interest here because it allows us to exclude general debilitation as a basis for the failure of rats with LPO lesions to increase operant rate following cellular dehydration, because their response rates were as high following the combined cellular and deprivation challenge as following water deprivation alone.

Operant response rates were then determined after 0 or 24 hours of water deprivation, combined with either acute cellular dehydration (injection of 1*M* NaCl, 1 percent of body weight) or control injections of isotonic NaCl. The control deprivation tests were administered first, followed by the combined challenges. Although this constraint raises the possibility of a trial sequence effect, it was more important to prevent the operant response from being conditioned to the injections per se, which would have contaminated baseline response rates (*10*).

Rats with LPO lesions, whether deprived of water or not, did not increase their operant rates when challenged with additional cellular dehydration (Table 1). Specifically, they only emitted four and ten more responses per session than in the baseline sessions (0 and 24 hours of deprivation, respectively). In contrast, the operant rates of normal rats increased by more than 270 percent in response to cellular dehydration alone and by more than 60 percent when cellular dehydration was superimposed on 24 hours of water deprivation. Cellular dehydration did not depress the operant rates of the brain-damaged rats (Table 1). Furthermore, two lines of evidence indicate that the lack of increase in operant rate in these animals cannot be attributed to a ceiling effect. (i) Imposing cellular dehydration did not increase operant rate in brain-damaged rats not deprived of water, even though this rate was less than half that obtained after 24 hours of water deprivation. (ii) After 48 hours of water deprivation, response rate in rats with lesions was 63 percent higher than after 24 hours of deprivation. Clearly, rats with LPO lesions could respond more frequently.

These data make clear that, at least in the case of drinking behavior, the urge to engage in a consummatory act can be divorced from actual consumption itself. Specifically, affect represents a summation of thirst afferents; thus, in an animal lacking the receptor to detect a change in body fluid economy, affect is correspondingly diminished. In contrast, the actual amount of water drunk in response to the combined challenge reflects the delay of cellular overhydration, is independent of a cellular thirst mechanism, and is therefore not directly related to the urge to drink.

Additional support for the separation of urge to drink from amount drunk is obtained from the drinking tests. If rate of drinking reflects urge and if termination of drinking is controlled, in large part, by the development of hyposmolality, then the following should hold: (i) After water deprivation alone, normal rats and those with LPO lesions should drink equivalent amounts by the end of the 5-hour test. This was borne out (Fig. 1). (ii) The initial drinking rate of deprived normal rats should be slightly higher than that of rats with LPO lesions because the normal rats can detect the modest cellular fluid loss that occurs during deprivation. This too occurred. Normal rats drank 2.06 ml per 100 g of body weight while rats with LPO lesions drank only 1.47 ml/100 g during the initial 15 minutes of drinking. (iii) Most important, the drinking rate of normal rats should be markedly accelerated when cellular dehydration is imposed on water deprivation, whereas this manipulation should not affect the drinking rate of rats with LPO lesions. This is precisely what happened. Following the combined challenge, normal rats drank 3.64 ml/100 g in the first 15 minutes, an increase of 1.58 ml/100 g above the amount consumed in response to deprivation alone. In contrast, rats with LPO lesions drank only 1.52 ml/100 g following the combined challenge as compared to 1.47 ml/100 g in response to deprivation alone (*11*).

In summary, we have shown that, in rats with LPO lesions, the urge to drink can be clearly distinguished from volume intake. Because the renal and body fluid dynamics of these rats, under the present testing conditions, were similar to those of normal rats (*12*), we suggest that this distinction can be extended to neurologically normal rats as well.

MARTIN H. TEICHER
ELLIOTT M. BLASS
Department of Psychology, Johns Hopkins University, Baltimore, Maryland

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6. Bilateral electrolytic lesions were made by passing 0.6 ma for 30 seconds through stainless steel electrodes guided, with the skull kept level, 7.3 mm below the dural surface, ± 1.5 mm lateral to the sagittal sinus, and 8.7 mm anterior to the intra-aural plane. After at least 7 days of recovery the rats were given a cellular dehydration challenge; those with water intake within the control range (95 percent confidence interval) were not used.
7. Overall analysis of variance showed significant differences between groups ($F = 5.17$; d.f. = 1, 18; $P < .05$) and a significant interaction between thirst challenges and groups ($F = 25.25$; d.f. = 2, 36; $P < .0001$) [one between-one within factor design analysis of variance (*8*, pp. 191–203)]. Post-hoc tests indicate that the only significant difference between control rats and those with LPO lesions occurred during the cellular dehydration challenge alone ($t = 3.35$, $P < .01$).
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12. Mean urine volumes and sodium concentrations 2

hours after injection of 1*M* NaCl (1 percent of body weight), in the absence of water, were 6.11 ml and 312.0 meq/liter in rats with lesions and 9.5 ml and 336.6 meq/liter in controls. At the end of the 5-hour combined-stimulus test, values in blood were as follows: sodium, 139.3 meq/liter in rats with lesions and 141.3 meq/liter in controls; hematocrit, 43.6 in animals with lesions and 42.8 in controls; osmolarity in plasma, 303.5 milliosmols in animals with lesions and 304.6 milliosmols in controls; and protein in plasma, 6.4 g/100 ml in animals with lesions and 6.7 g/100 ml in controls.

3 September 1975; revised 31 October 1975

Human Y-Chromosome-Specific Reiterated DNA

Abstract. *Radiolabeled reiterated DNA specific for the human Y chromosome has been obtained by extensive reassociations between [³H]DNA prepared from men and excess DNA from women. These highly purified labeled sequences reassociate only with DNA from individuals with a Y chromosome. The percentage of Y-chromosome-specific DNA isolated from individuals with differing numbers of Y chromosomes is a function of the number of Y chromosomes present. The purified Y-chromosome-specific sequences may represent between 7 and 11 percent of the human Y chromosome.*

The mechanisms by which the Y chromosome engenders maleness are unknown. Investigation has been handicapped by the lack of Y-chromosome-specific gene assignments. In this report we describe the isolation of ³H-labeled reiterated DNA unique to the human Y chromosome.

Whole blood lymphocytes obtained from individuals bearing the chromosome constitution 46,XX; 46,XY; 47,XYY; or 48,XYYY were labeled continuously in cultures containing [³H]thymidine (*1*). DNA isolated (*2*) from each of these cultures was sonicated (*3*), and reiterated sequences were enriched by reassociation to C_0t value of 46 (*4*). The reassociated reiterated sequences, representing 30 to 40 percent of the genome, were collected on hydroxylapatite and subsequently fractionated by stepwise elution with increasing concentrations of phosphate buffer, *p*H 6.8 (*5*). Preliminary experiments indicated that the most stable duplexes, that is, those eluted by phosphate concentrations between 0.25 and 0.3*M*, were slightly enriched for DNA sequences of the Y chromosome. These stable duplexes were mixed with a 5000-fold excess of unlabeled DNA from a 46,XX woman. The mixture was incubated to C_0t 460. Those sequences failing to reassociate and therefore not binding to hydroxylapatite were collected and assayed (*6*) for Y-chromosome-specific sequences. For each individual, the percentage of ³H-labeled Y-chromosome-specific sequences present in the entire genome was calculated as the arithmetic product of the amount of ³H-labeled DNA recovered after each step, and the percent of the final fraction assayed (*6*) as Y-chromosome-specific (Table 1). As seen, this percentage is a linear function of the number of Y chromosomes present in each subject. Despite low recovery of stable duplexes from the DNA of the 46,XY subject and consequently a poor fit to linearity in this instance, the overall correlation coefficient of the regression of the percentage of Y-chromosome-specific DNA on Y-chromosome dose is .99.

These results (Table 1) establish that Y-chromosome-specific sequences indeed exist. Their further purification was facilitated by an increase in labeling over that attainable in lymphocyte culture. This was achieved by using the nick translation function (*7*) of DNA polymerase I (*Micrococcus luteus*) (Miles Laboratories, Inc.) to incorporate [³H]TTP (thymidine triphosphate) (New England Nuclear, 40 to 60 c/mmole) into isolated DNA from a 47,XYY individual. Prior to labeling, reiterated DNA from this subject was prepared by reassociation to C_0t equal to 46. Three percent of the genome was isolated as stable duplexes by elution with high salt from hydroxylapatite and then nick-translated. The specific activity of products ranged from 5×10^7 to 1×10^8 dpm/μg. The labeled product was reassociated to a whole genome C_0t of 46. Fifty percent of this product formed duplexes, which were collected on hydroxylapatite. The eluted duplexes were subsequently reassociated with a 40,000-fold excess of unlabeled DNA from a 46,XX subject. Those sequences which failed to reassociate, that is, 20 percent of material entering this step, were exposed to a second challenge with excess DNA from a 46,XX individual. ³H-Labeled sequences which again failed to reassociate (73 percent of input) were assayed (*6*) for Y-chromosome specificity. The portion of reiterated labeled DNA that remained reassociable was now completely specific for the Y chromosome (Fig.

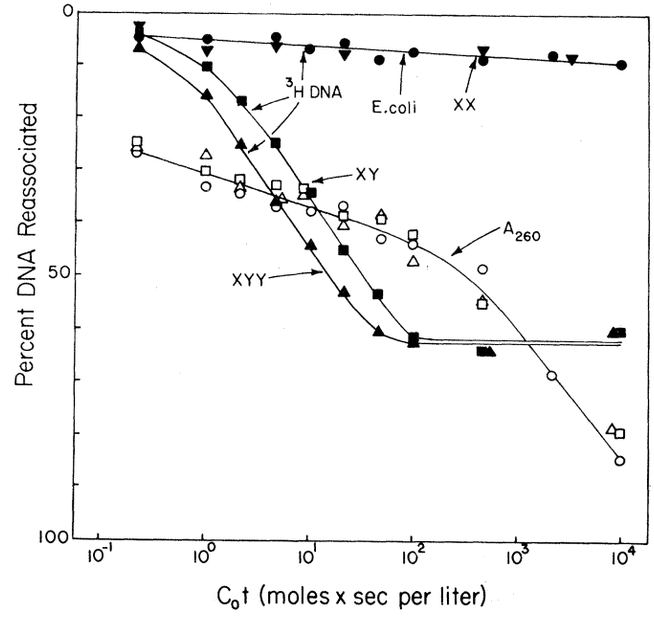

Fig. 1. Reassociation curves between a fraction of ³H-labeled DNA from a 47,XYY individual and excess whole genome DNA from various sources. The fraction of DNA from the XYY individual represents the stable duplexes isolated from hydroxylapatite after reassociation to C_0t 46 (*4*), thereafter labeled with [³H]TTP by nick translation (*7*), and then twice challenged with excess whole genome DNA from a 46,XX individual. The ³H-labeled sequences that twice failed to reassociate when challenged were then reassociated, as shown here, with more than a 10,000-fold excess of whole genome DNA from 46,XY ■—■, 47,XYY ▲—▲, 46,XX ●—● individuals, and *Escherichia coli* ▼—▼. Reassociation of labeled sequences was analyzed radiometrically. Reassociation of the unlabeled whole genome human DNA, monitored optically at A_{260}, is indicated by the open symbols: 46,XY □—□, 47,XYY △—△, and 46,XX ○—○. The analyses of *E. coli* DNA at A_{260} are not shown; it reassociated to approximately 90 percent with a $C_0t_{1/2}$ of 4. Reassociation conditions were the same as described in (*6*).