fish were upside down or pulled to the surface; stage II was reached when the fish swam continuously at an angle of 45° to the horizontal; and stage III was reached when the fish swam normally. The fish were subjected to 3 trials: Trial 1 (day 1) for 180 min, trial 2 (day 2) for 90 min, and trial 3 (day 6) for 120 min. The percentage trained was plotted against time for each 15-min-period during the training period for each trial.

Results. The effect of acclimation on learning and retention scores on the 5 groups of fish is shown in the figure. Fish acclimated at 21 °C all reached 100% trained after 150 min in trial 1, 75 min in trial 2 and 105 min in trial 3. The fact that the fish learned best on trial 2 and reached 100% trained on trial 3 in a time intermediate between trials 1 and 2, indicates a memory component.

The cold acclimated fish failed to learn the task. The fish remained floating upside down, but occasionally swam upside down. Fish that had previously been acclimated to 5 °C for 3 weeks and then placed in water at 21 °C, 48 h before the first trial, learned the task in 90 min on day 1. The scores for this group of fish were similar to the fish acclimated at 21 °C during trial 2 and slightly better at trial 3.

Fish acclimated at 33 °C failed to learn the task during any one of the 3 trials, and those transferred from 33 °C to 21 °C still failed to learn the task even 1 week after being transferred from the warm temperature. These results agree with those previously reported by Shashoua for summer fish[3].

Discussion. Shashoua[3] found that goldfish learn the task used in this experiment better in winter than in summer. The previous water temperature at which the fish were kept a few days before the experiments was not mentioned. Our results have shown that fish taken from a warm water environment and trained at room temperature still fail to learn the swimming skill 8 days after the transfer. Changes in biogenic amine levels[4], as well as changes in steroid levels with the onset of the spawning season[3], have been cited by Shashoua as reasons for these seasonal variations in learning. Control for the direct temperature effects was not, however, carried out.

French[6] has tested the effect of the acclimation temperature on the retention of a learned maze performance in goldfish. All the fish were tested at 16 °C. Fish acclimatized at low temperatures exhibited increased retention of the maze habit. These results, which involved transferring fish from cold to warmer water are in agreement with our findings that learning is improved when fish are transferred from 5 °C to 21 °C. Furthermore, French also found that significantly more errors were made by fish kept at 28 °C than fish at 16 or 4 °C[10], which is also in agreement with the results we have found for warm acclimated groups of fish.

The poor results of cold acclimated (5 °C) fish when they were not transferred to 21 °C are probably due to the low temperature slowing down metabolic reactions and altering neural and muscle function.

The present paper, in which sexually immature carp fingerlings were used, indicates that temperature acclimation is a significant factor in learning and memory. Although temperature is known to affect ionic composition[7], amino acid levels[8], oxygen consumption[9], biogenic amine levels[4], hormonal levels and nervous system functions[10], the exact manner in which memory and learning are affected by these changes is not quite clear.

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Positive after-image, PAI: Early erasure by saccadic eye movement or Jendrassik manoeuvre[1]

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Summary. Positive after-images (PAIs) evoked by low intensity stroboscopic flash are erased by voluntary large amplitude saccadic eye movements. The duration of the PAI is shortened by a Jendrassik manoeuvre. The results suggest that muscle spindle afferent impulses conducted centrally are involved in the erasure phenomenon. The duration of the PAI is modifiable by drugs. The social implications of PAI are considered briefly.

A flash of stroboscopic light evokes a series of images that can be examined subjectively. The real image observed during the flash fades rapidly, disappears and is replaced by a highly-detailed positive after-image, PAI, the onset and duration of which is dependent upon the intensity of the stimulating light. This image fades to a uniform neutral PAI in which the detail is absent, and is followed by a more durable negative after-image[2–4]. This communication reports that the PAI is erased by a large amplitude saccadic eye movement. This striking phenomenon can be produced easily by simply flashing a strobe light, photostimulator or electronic flashgun of low intensity while observing targets containing detailed images. It is suggested that the phenomenon of erasure is initiated by afferent impulses from activated muscle spindle receptors on the extraocular muscles which are processed centrally.

Methods. The subject, head in a fixed position, seated comfortably and allowed to adapt for 15 min to a completely darkened room, faces a target surrounded by a black nonreflecting surface. The target consists of a series of black and white concentric rings which subtend a solid angle of 10° at a distance of 35 cm.

A Grass Photostimulator, Model PS22, placed out of view of the subject at a height of 75 cm above the target which is tilted at an angle of 45° from the horizontal toward the subject, is triggered every 15 sec by a Grass

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Duration of positive after-image as function of flash intensity, saccadic movement and Jendrassik manoeuvre

Flash intensity at source Level	Candlepower	Procedure	PAI duration ± SD (sec) J. S., age 28*	A. F., age 49**
I	9.20×10^4		1.43 ± 0.15	1.61 ± 0.04
II	1.85×10^5		1.93 ± 0.06	2.09 ± 0.13
III	3.75×10^5		2.09 ± 0.05	1.94 ± 0.14
IV	7.50×10^5		2.40 ± 0.10	2.30 ± 0.10
V	1.50×10^6		3.54 ± 0.10	2.97 ± 0.10
III	3.75×10^5	a) Control	2.70 ± 0.07	2.27 ± 0.19
III	3.75×10^5	b) Saccadic movement	1.21 ± 0.35***	1.19 ± 0.16***
III	3.75×10^5	c) Control	–	2.15 ± 0.16
III	3.75×10^5	d) Jendrassik manoeuvre	–	1.58 ± 0.18***
		Ratio b/a	0.448	0.524
		Ratio d/c	–	0.735

*Each point is the average of 3 trials. **Each point is the average of 4–6 trials. ***$p < 0.05$.

S88 stimulator to produce pulses of white light of about 10 μsec duration over a 5 logarithmic step intensity range of from 9.2×10^4 to 1.5×10^6 candlepower. Onset and duration of PAIs are quantified over this intensity range by having the subject activate a timer for the duration of the PAI by means of a microswitch. Responses are quantified after a series of training trials. Quantification problems arise from subjects' interpretation of endpoints, reaction time, partly or completely obliterating targets by blinking, anticipatory responses, inattentiveness, peripheral rather than foveal viewing and after-images that seem to move. Training eliminates most of these problems.

Onset and duration of PAI are measured over the 5 step series of intensities. An intermediate intensity of stimulation is selected and the subject is asked to make a rapid maximal saccadic movement (from central fixation to far left, to far right, back to central fixation) when the PAI first appears. He reports the status of the PAI without being told what to expect from the saccadic movement. The response is compared with the control response. Some subjects are asked to perform a Jendrassik manoeuvre, i.e., reinforcement of the stretch reflex activity by clasping their hands and pulling them against each to activate the gamma efferent fusimotor system. Onset and duration of the PAI after fusimotor activation is compared with control performances conducted at the same light intensity.

Results. The PAI onset, duration and erasure phenomenon was studied in 28 subjects of both sexes ranging in age from 8 to 59 years. A positive after-image was detected by all subjects whether trained or naive; 27 of 28 reported the erasure phenomenon. Some reported a moderate amount of tension ('sensation of effort') developed when they attempted to fixate an invisible target. Others noted that the PAI drifted.

The table demonstrates that the duration of PAI is directly proportional to the light intensity, attaining a value at the highest intensity that was twice that at the lowest intensity. Onset time itself, for a particular subject, does not change significantly over the stimulus range, but varies considerably from subject to subject, e.g., at 3.75×10^5 candlepower onset time for J. S., male, age 28, was 0.45 ± 0.26 sec as compared to 1.13 ± 0.29 sec for A. F., male, age 49. The PAI duration is significantly shortened by large amplitude saccades (55.2% and 47.6% for J. S. and A. F., respectively, table). The cited examples are typical. In the case of children, the PAI at a given stimulus intensity tended to be longer than in adults, e.g., at stimulus intensity I, S. H., male, age 8, and S. M., female, age 11, displayed PAIs of 3.06 ± 0.19 and 2.45 ± 0.08 sec, respectively. PAI duration was significantly decreased by a Jendrassik manoeuvre. This procedure, usually, was less effective than a saccade (table, compare b/a ratio with d/c ratio for subject A. F.). 2 drug related effects on PAI duration were noted. After coffee (containing about 100 mg of caffeine), in male subject M.A., age 22, the saccade-induced reduction of PAI duration was shortened by more than 60%, initially, then returned to levels more characteristic for this subject within 1 h. Subject D. G., male, age 26, who recently was placed on 0.3 mg/day of levothyroxine sodium therapy exhibited PAIs of greatly reduced duration at all stimulus intensities (e.g. at a stimulus intensity of 1.5×10^6 candlepower his PAI duration was 1.63 ± 0.19 sec, although the onset time was similar to that of drug-free subjects, viz., 0.83 ± 0.23 sec).

Discussion. According to Craik[5] positive and negative after-images originate in the retina from photochemical events occurring at the receptor as the result of stimulation by light. Beyond the receptors, retinal processing involves both inhibitory and excitatory events[6,7]. Pickering and Varju[6] recording from the optic tectum of animals with intact optic tracts, and Sickel and Crescitelli[8] from the isolated frog retina found that a brief light stimulus initially evoked spiking, then a silent period or delayed-off response followed by prolonged spiking. This suggests an interplay between ongoing excitatory and inhibitory processes and according to these investigators, a possible involvement with after-images. Riggs et al.[2], examined suppression of the electrically-evoked visual phosphenes during saccadic movement and found that the threshold was raised during eye movement indicating the participation of neural elements in this phenomenon since the optical system was bypassed. For a recent, comprehensive treatment of the subject of saccadic suppression see Matin[9].

The shortening of the duration of the PAI by saccadic movement or Jendrassik manoeuvre in the present study appears to indicate involvement of afferent pathways from extraocular muscle spindles conducting impulses centrally supporting a hypothesis first suggested in 1903[10]. In man, extraocular muscle spindles are in abundance

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and their afferent impulses can be recorded in the superior colliculus as well as in other brain loci involved with eye movements[11]. Cooper et al.[11] also found evidence of limb proprioceptor afferent connections in the same brain areas. Although Pickering and Varju[6] stated that optic nerve efferents do not contribute to the delayed-off responses, the present study suggests the possibility that the processing of after-images is modifiable centrally. Furthermore, the Popovs[12] have demonstrated that after-images can be obtained by conditioned reflex responses to sound!

Graham and Pong[7] hypothesize feedback loops within the amacrine-bipolar dyads of the retina which regulate ganglion cell output and are mediated through GABA-minergic systems. The time-course of GABA depolarization in primary afferent muscle spindle neurons[13], bears temporal resemblance to the positive after-image time-course resulting from ultra-short light stimuli. GABA, however, is only 1 of a number of putative inhibitory neurotransmitters present in the visual and central nervous system[14]. The present study of positive after-images and their erasure by saccadic movement suggests that there is an apparently time-linked latching mechanism that is unmasked by very brief light stimuli. The latching mechanism possibly causes storage of the transmitted retinal image in the visual cortex or at an intermediate locus. Under steady state illumination it maintains a stationary image while the micro-saccadic activity of the eye involves other receptors to fix the same point in space. The concomitant latching and unlatching through the intervention of excitatory and inhibitory neurotransmitters is perhaps the 'neuronic shutter mechanism' proposed by Lindsley[15] to explain why one perceives images while scanning that are stable and stationary, with clarity.

A 2-sec PAI resulting from a 10-μsec stimulus represents an amplification factor of 2×10^5. This finding has far-reaching implications with respect to subliminal perception, education and even thought control.

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GABAergic inhibition of neurons in the ventral tegmental area

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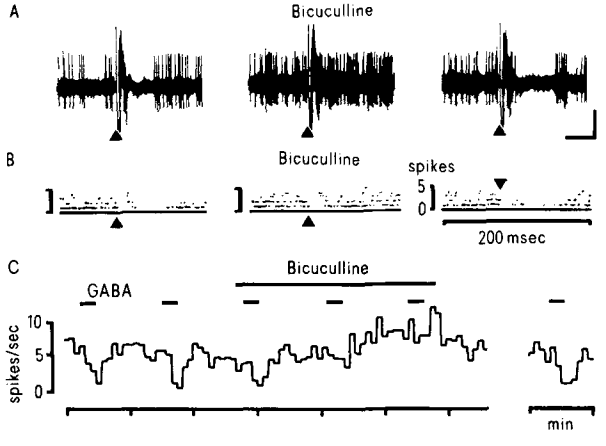
Summary. Stimulation of the nucleus accumbens evokes a potent inhibition in neurons of the ventral tegmental area. GABA is likely to act as a transmitter in this descending inhibitory system.

Neurons originating in the ventral tegmental area (VTA) have diffuse projections to subcortical limbic structures[1,2]. This mesolimbic system has recently received considerable attention because of its possible involvement in the pathogenesis of schizophrenia[1,3]. Biochemical[3] and behavioral[4] experiments have provided indirect evidence to suggest that there is a preferential inhibitory modulation of VTA neurons by GABAergic pathways descending from the limbic forebrain. This possibility was examined more directly in the present study by using microelectrode recording and microiontophoretic techniques.

Materials and methods. 10 male rats (200–400 g) were anesthetized with urethane (1.3 g/kg i.p.) and mounted in a stereotaxic frame. Multibarrel glass micropipettes (2–4 barrels, 3–5 μm tip diameter) were used for recording and microiontophoretic application of the following substances: gamma-aminobutyric acid (GABA, 0.5 M, pH 3.5), bicuculline methiodide (20 mM in 165 mM NaCl, pH 3.5) and pontamine sky blue (2% in 0.5 M acetic acid). The micropipettes were placed stereotaxically in the VTA using the atlas of De Groot[5] and the location of the micropipettes was marked by ejecting pontamine sky blue from the tips. The nucleus accumbens septi was stimulated by small bipolar electrodes which were also used to make electrolytic lesions at the termination of the experiments to verify the stimulation sites.

Results and discussion. Stimulation of the nucleus accumbens (1–20 V, 0.2–0.5 msec) evoked patterns of inhibition and excitation in neurons of the VTA similar to those



A Inhibition of a VTA neuron by stimulation of the nucleus accumbens (left trace), antagonized by iontophoretically applied bicuculline methiodide (100 nA, 3 min, middle trace) and recovery of the inhibition 6 min after withdrawal of bicuculline. 6 superimposed sweeps. Triangles mark the time of stimulation of the nucleus accumbens (10 V, 0.5 msec). Calibration: 40 msec; 1 mV.
B Same neuron as in *A*: peristimulus-time-histograms. 64 sweeps, 205 bins, 200 msec duration.
C Reversible block of depressant actions of GABA (40 nA) by microiontophoretically applied bicuculline methiodide (50 nA). Gap represents 6 min.

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