with ^{125}I-labeled T lymphocytes (data not shown).

Comparison of the molecular size determined for T4 62,000-dalton with that of the Lyt 1.1 antigens from murine thymocytes shows a striking similarity. Alloantiserum to Lyt 1.1 precipitated a 67,000-dalton glycoprotein and an 87,000-dalton glycoprotein that could be labeled with tritiated sodium borohydride and galactose oxidase but not with the ^{125}I-labeled lactoperoxidase (*9*). A monoclonal xenoantiserum [termed 53-7.3 (*10*)] detected a 70,000-dalton glycoprotein on ^{125}I-labeled C57B1/6 thymocytes (*10*).

Murine Lyt 2 and Lyt 3 antigens were found on cell surface glycoproteins from thymocytes labeled with ^{125}I-labeled lactoperoxidase (*10, 11*). Immune precipitations carried out with alloantiserums to Lyt 2 and Lyt 3 detected a 35,000-dalton protein for each marker on C57B1/6 thymocytes (*11*). However, in contrast, a monoclonal rat antibody (53-6.7) precipitated a complex of two subunits of approximately 30,000 and 35,000 daltons that was resolved under reducing conditions (*10*). Under nonreducing conditions, a 65,000-dalton glycoprotein was found. These molecular sizes are similar to those determined for T5 (30,000 to 32,000 daltons).

Our results plus the previous functional data indicate that the glycoproteins recognized by the monoclonal antiserums to T4 and T5, respectively, are the human homologs of the Lyt 1 and Lyt 2,3 antigens. Isolation and further characterization of these cell surface markers will be important in determining the precise role of T4 and T5 in the functions of the cells that express them. Information obtained in such studies would also aid in our understanding of the differentiative pathways of human T lymphocytes.

COX TERHORST, ANDRE VAN AGTHOVEN
ELLIS REINHERZ, STUART SCHLOSSMAN
Sidney Farber Cancer Institute,
Harvard Medical School,
Boston, Massachusetts 02115

References and Notes

1. E. Reinherz and S. Schlossman, *Cell*, in press.
2. E. L. Reinherz, P. C. Kung, G. Goldstein, S. F. Schlossman, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4061 (1979).
3. _____, *J. Immunol.*, in press.
4. R. L. Evans, H. Lazarus, A. Penta, S. F. Schlossman, *J. Immunol.* **120**, 1423 (1978); E. L. Reinherz and S. F. Schlossman, *ibid.* **122**, 1335 (1979).
5. H. Cantor and E. A. Boyse, *Cold Spring Harbor Symp. Quant. Biol.* **51**, 23 (1977).
6. C. G. Gahmberg and L. C. Anderson, *Ann. N.Y. Acad. Sci.* **312**, 240 (1978).
7. P. Cresswell, T. Springer, J. L. Strominger, M. J. Turner, H. M. Grey, R. T. Kubo, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2123 (1974).
8. J. Michaelson, L. Flaherty, E. Vitetta, M. D. Poulik, *J. Exp. Med.* **145**, 1066 (1977).
9. P. J. Durda, C. Shapiro, P. D. Gottlieb, *J. Immunol.* **120**, 53 (1978).
10. J. A. Ledbetter and L. A. Herzenberg, *Immunol. Rev.* **47**, 65 (1979).
11. P. J. Durda and P. D. Gottlieb, *J. Exp. Med.* **144**, 476 (1976).
12. A. Boyum, *Scand. J. Clin. Lab. Invest. Suppl.* **21**, 97 (1968).
13. U. K. Laemmli, *Nature (London)* **227**, 680 (1970).
14. W. M. Bonner and R. A. Laskey, *Eur. J. Biochem.* **46**, 83 (1974).
15. C. Terhorst, R. Robb, C. Jones, J. L. Strominger, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4002 (1977).
16. We thank J. Distaso and K. LeClair for technical assistance. The antiserum to β2m was from Dr. L. Nadler (Sidney Farber Cancer Institute). Supported by NIH grant AI 15066 and by a travel fellowship (to A.vanA.) from the Netherlands Foundation for the Advancement of Pure Research (Z.W.O.).

21 March 1980

Excitatory and Inhibitory Effects of Serotonin on Sensorimotor Reactivity Measured with Acoustic Startle

Abstract. *Serotonin infused into the lateral ventricle in rats produced a dose-dependent depression of the acoustic startle reflex. When infused onto the spinal cord, serotonin produced a dose-dependent increase in startle. Thus the same neurotransmitter can modulate the same behavior in opposite ways, depending on which part of the central nervous system is involved.*

Serotonin is often considered to be an important behavioral inhibitor (*1*). However, there are data that are difficult to reconcile with this conclusion (*2*). The acoustic startle response, a simple reflex behavior, is modified by changes in serotonin levels. Small amounts of serotonin or serotonin agonists, infused directly into the hippocampus (*3*) or the ventricles (*4*), depress startle (*5*), suggesting that serotonin inhibits this behavior. However, markedly increasing the levels of serotonin in the brain and spinal cord (*6, 7*) or administering drugs that mimic serotonin, heightens startle (*8*), suggesting that serotonin is excitatory.

Excitatory or inhibitory effects of neurochemicals on behavior may depend on the location and nature of the receptors involved. We now report that serotonin depresses acoustic startle when infused into the forebrain (intraventricularly), but heightens this response when infused onto the spinal cord (intrathecally).

Male albino rats (300 to 400 g) were anesthetized with chloral hydrate and implanted with cannulas into the lateral ventricles or with catheters into the spinal cord (*9*). Twenty-four hours later, an injection of the monoamine oxidase inhibitor pargyline (25 mg/kg) was given (*10*). One hour later, the rats were placed in a startle apparatus and subjected to noise bursts every 20 seconds for 15 minutes (*11*). The animals were then infused with various doses of serotonin dissolved in saline (*p*H 7.4). The animals with cannulated lateral ventricles received saline or 0.78, 3.12, 12.5, 50, or 200 μg of serotonin. The spinal animals received saline or 12.5, 25, 50, 100, or 200 μg of serotonin. (Doses are based on the salt weight of serotonin creatinine sulfate.) There were three rats in each of the experimental and control groups. A total of 10 μl of fluid was administered to each animal at the rate of 4 μl/min (*12*). The rats were returned immediately to the test chamber and given noise bursts every 20 seconds for 20 minutes. Next, the rats with cannulated ventricles were infused with 10 μl of 0.5 percent Fast Green FCF dye. Fifteen minutes later they were perfused, and their brains were examined to ensure adequate infusion of the dye. Animals in which one or both ventricles were incompletely infused (8 percent) or whose catheters showed signs of being clogged (17 percent) were not included in the data.

Figure 1 shows that serotonin caused a rapid decrease in startle amplitude when infused into the lateral ventricle and a rapid increase when infused onto the spinal cord. As shown in Fig. 2, both of these effects were directly related to the amount of serotonin infused. An overall analysis of variance with doses common to both placements (saline and 12.5, 50, and 200 μg of serotonin) revealed a significant dose × placement interaction, F (3, 12) = 7.14, $P < .01$. Subsequent analyses indicated a dose-related depression of startle when serotonin was infused into the lateral ventricle, linear F (1, 12) = 20.04, $P < .001$, and a dose-related excitation of startle when serotonin was infused onto the spinal cord, linear F (1, 12) = 17.35, $P < .001$ (*13*).

Infusion of serotonin into the two areas also had opposite effects on other types of motor activity. Doses ($\geq$ 3.12 μg) that depressed startle when given intraventricularly produced catalepsy (*14*). In contrast, doses that increased startle when given intrathecally produced tremor of the hind quarters, indicative of a localized serotonin "syndrome" (*9, 15*).

Recent single-unit recording studies indicate that there are different types of

0036-8075/80/0725-0521$00.50/0

serotonin receptors in the rat central nervous system (*16, 17*). One type is found in certain forebrain structures. Here serotonin depresses neuronal firing when applied microiontophoretically. Another type is found in the brainstem and spinal cord. Here serotonin facilitates the excitatory inputs of other neurotransmitters. Classical serotonin antagonists attenuate the facilitatory effects of serotonin in the brainstem and spinal cord but fail to alter the inhibitory effects in the forebrain. These data predict that a serotonin antagonist would attenuate the excitatory effects of intrathecally administered serotonin on startle but would not block the inhibitory effects of intraventricularly administered serotonin.

To test this hypothesis, ten rats were fitted with intrathecal catheters and ten with intraventricular cannulas. On the next day, all the rats were injected with pargyline (25 mg/kg) and 1 hour later were given noise bursts every 20 seconds for 15 minutes. Half of each group was then injected intraperitoneally with saline, and half with the serotonin antagonist cinanserin (10 mg/kg). The rats were immediately given another 15-minute series of noise bursts. Finally, the animals were infused with 100 μg of serotonin intrathecally or 12.5 μg intraventricularly and then subjected to noise bursts for the next 20 minutes. Consistent with predictions based on single-unit studies, cinanserin significantly attenuated the excitatory effect of intrathecally given serotonin (55 versus 257 percent, $P < .05$) but did not block the depressant effect of intraventricularly given serotonin (−88 versus −72 percent), even though a lower amount of serotonin was administered intraventricularly.

Our data indicate that a simple reflex behavior can be modulated in opposite directions by the same neurotransmitter, depending on where the neurotransmitter acts in the central nervous system. The inhibitory effects are probably mediated by structures that surround the lateral ventricles or cerebral aqueduct, since radioactive serotonin infused into the lateral ventricles seems to reach sites in these areas only and is not distributed throughout the cerebrospinal fluid space, probably because it is actively taken up from cerebrospinal fluid by serotonin terminals before it can diffuse very far (*18*). The excitatory effects are probably mediated by a facilitation of the lower motor neuron's response to the excitatory volley initiated by the startle stimulus—serotonin has been shown to facilitate lower motor neuron responsivity to afferent stimulation (*17*).

The finding that forebrain and spinal serotonin affect startle in opposite ways has a number of implications. First, it can no longer be assumed that a neurochemical modulates a behavior in a simple, unidirectional fashion. There may be a critical balance between the inhibitory and excitatory effects of serotonin and other neurotransmitters on behavior. Procedures that change absolute levels of serotonin throughout the central nervous system may not actually change this balance, and hence fail to alter behavior markedly (*7*).

Second, these data indicate that the spinal cord is a possible target organ for mediating the effects of drugs on behavior. Generally, it is assumed that when a drug alters behavior it does so by affecting the brain, but our data suggest that some of the effects seen after systemic administration of a drug may be mediated partially or even entirely in the spinal cord. This finding is particularly important in view of the fact that the spinal cord is the final common pathway for most behaviors measured in psychopharmacological studies (avoidance conditioning, bar pressing, locomotor activity, and stereotyped behavior).

Third, serotonin may exercise reciprocal control over behaviors other than the startle reflex. For example, it has repeatedly been implicated in the control of mood (*19*). However, reported correlations between levels of serotonin metabolites in cerebrospinal fluid and psychiatric symptomatology may be more reflective of spinal serotonin metabolites associated with motor activity than with mood, since much of the fluid obtained in these studies was of spinal or lower brainstem origin (*20*).

Finally, the wide shifts in mood seen in bipolar depressive illness may reflect an imbalance within the serotonin system from one extreme to the other. It would be of considerable interest, therefore, to determine how drugs, like lithium or tricyclic antidepressants, which are relatively successful clinically and which alter serotonin transmission, af-

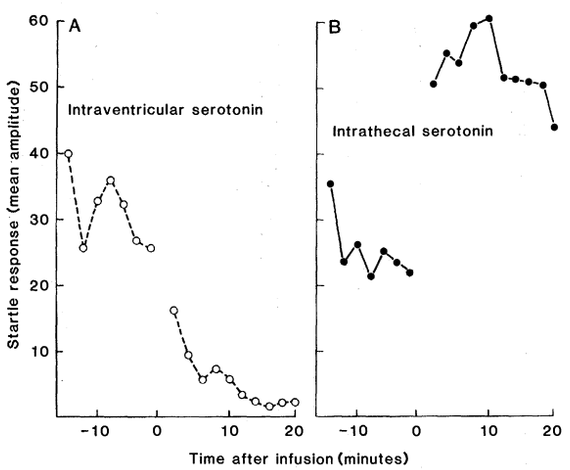

Fig. 1. Mean amplitude of startle over 2-minute periods before and after infusion (time 0) of 200 μg of serotonin into the lateral ventricle (A) or onto the spinal cord (B).

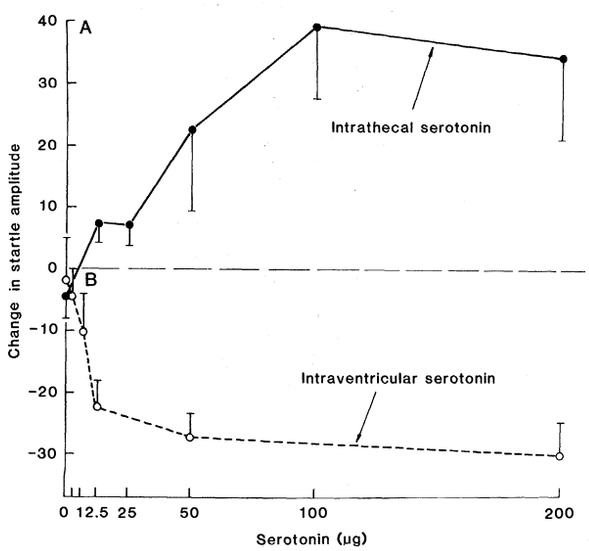

Fig. 2. Mean change in startle, computed as the mean amplitude of startle during the 20-minute period after infusion minus the mean amplitude of startle during the 15-minute period before infusion, for each dose of serotonin infused (A) onto the spinal cord or (B) into the lateral ventricle (± standard error).

fect the balance between inhibitory and excitatory effects of serotonin on behavior. The present model might be useful for testing such interactions.

MICHAEL DAVIS
DAVID I. ASTRACHAN
ELIZABETH KASS

Department of Psychiatry, Yale University School of Medicine and Connecticut Mental Health Center, New Haven 06508

References and Notes

1. J. B. Meyerson and M. Eliasson, *Handbook of Psychopharmacology*, vol. 8, *Drugs, Neurotransmitters, and Behavior* (Plenum, New York, 1977); L. S. Seiden and L. A. Dykstra, *Psychopharmacology: A Biochemical and Behavioral Approach* (Van Nostrand Reinhold, New York, 1977); M. H. Sheard and M. Davis, *Eur. J. Pharmacol.* **40**, 295 (1976).
2. S. Barasi and M. H. T. Roberts, *J. Physiol. (London)* **236**, 11 (1976); J. Maj, W. Palider, L. Baran, *J. Neural Transm.* **38**, 131 (1976); N. R. Myslinski and E. G. Anderson, *J. Pharmacol. Exp. Ther.* **204**, 19 (1978); B. L. Jacobs, *Life Sci.* **19**, 77 (1976); D. G. Grahame-Smith, *Br. J. Pharmacol.* **43**, 856 (1971).
3. M. A. Geyer, *Psychopharmacol. Commun.* **1**, 675 (1975).
4. M. A. Geyer, A. J. D. Warbritton, D. B. Menkes, J. A. Zook, A. J. Mandel, *Pharmacol. Biochem. Behav.* **3**, 293 (1975).
5. M. Davis and M. H. Sheard, *Eur. J. Pharmacol.* **35**, 261 (1976); *Pharmacol. Biochem. Behav.* **2**, 827 (1974); M. Davis and J. K. Walters, *ibid.* **6**, 427 (1977).
6. L. D. Fechter, *Pharmacol. Biochem. Behav.* **2**, 161 (1974).
7. M. Davis, *Neurosci. Biobehav. Rev.*, in press.
8. _____, D. I. Astrachan, P. M. Gendelman, D. S. Gendelman, *Psychopharmacology*, in press.
9. Cannulas were made from 23-gauge hypodermic needles turned down in a metal lathe to a hub 8 mm long and 4 mm wide, and were threaded inside to accept inner or infusion cannulas. Inner and infusion cannulas were made from 30-gauge hypodermic needles turned down and threaded to fit into the outer cannulas so that their tips protruded 1.5 mm beyond the outer tips. The cannulas were implanted 1.4 mm lateral to the lambda and 4 mm below the top of the skull in rats anesthetized with chloral hydrate, and were secured with skull screws and dental cement. Intrathecal catheters were made from PE 10 polyethylene tubing, as described by T. L. Yaksh and P. R. Wilson [*J. Pharmacol. Exp. Ther.* **208**, 446 (1979)].
10. In intact animals, excitatory motor effects caused by serotonin or its precursors occur only in the presence of a monoamine oxidase inhibitor. Therefore the rats were first treated with a moderate dose of pargyline, which preliminary studies indicated does not alter baseline startle levels. In order to equate conditions for both lateral ventricle and spinal placements, pargyline was given to all animals. After completing the dose-response curves, we found, however, that higher amounts of serotonin (200 to 400 μg) heightened the startle response when given intrathecally without pargyline pretreatment. Since Geyer *et al.* (*4*) already showed that serotonin given alone intraventricularly depresses startle, pretreatment with a monoamine oxidase inhibitor is not required to demonstrate forebrain versus spinal effects of serotonin on startle.
11. The apparatus used to measure startle is described by G. T. Weiss and M. Davis [*Pharmacol. Biochem. Behav.* **4**, 713 (1976)]. Briefly, five separate stabilimeters were used to record the amplitude of the startle response. Each stabilimeter consisted of an 8 by 15 by 15 cm Plexiglas and wire mesh cage suspended between compression springs within a steel frame. Cage movement caused displacement of an accelerometer; the resultant voltage was proportional to the velocity of displacement. Startle amplitude was defined as the maximum accelerometer voltage during the first 200 msec after the stimulus and was measured with a sample-and-hold circuit. The stabilimeters were housed in a dimly lit, ventilated, sound-attenuated chamber 1.1 m from a high-frequency speaker. The startle stimulus was a 90-msec, 115-dB burst of white noise with a rise-decay time of 5 msec. Background white noise, provided by a white noise generator, was 46 dB. Sound level measurements were made in the cages with a General Radio model 1551-C sound level meter (A scale).
12. M. Davis and R. D'Aquila, *Pharmacol. Biochem. Behav.* **4**, 469 (1976).
13. B. J. Weiner, *Statistical Principles in Experimental Design* (McGraw-Hill, New York, 1962), pp. 70–77.
14. Catalepsy was measured as described by P. Worms and K. G. Lloyd [*Pharmacological Methods in Toxicology* (Raven, London, 1979)].
15. B. L. Jacobs (*2*). However, the increase in startle cannot be explained by an artifact caused by the serotonin syndrome and mistaken for startle since measuring cage movement in the absence of a startle stimulus revealed that the amount of drug-induced movement recorded in this way was far below that recorded in the presence of the noise bursts.
16. G. K. Aghajanian and H. J. Haigler, *Proceedings, Fifth International Congress of Pharmacology* (Basel Press, Switzerland, 1972); R. B. McCall and G. K. Aghajanian, *Brain Res.* **169**, 11 (1979).
17. R. S. Neuman and S. R. White, *Brain Res.*, in press.
18. K. Fuxe, T. Hokfelt, M. Ritzen, U. Ungerstedt, *Histochemie* **16**, 186 (1968).
19. T. N. Chase and D. L. Murphy, *Annu. Rev., Pharmacol.* **13**, 181 (1973); D. L. Murphy, I. Campbell, J. L. Costa, *Psychopharmacology: A Generation of Progress* (Raven, New York, 1978).
20. E. Garelis, S. N. Young, S. Lal, T. L. Sourkes, *Brain Res.* **79**, 1 (1974).
21. This research was supported by NSF grant BMS-78-04170, NIMH grants MH-25642 and MH-18949, research scientist development award MH-00004 to M.D., and by the state of Connecticut.

8 February 1980

Female Mate Choice in a Neotropical Frog

Abstract. *Female* Physalaemus pustulosus *choose their mates and are more likely to choose larger males. There is a significant negative correlation between the size of the male and the fundamental frequency of one of the components of its advertisement call. Playback experiments demonstrate that females are capable of choosing larger males by distinguishing among differences in spectral components of the advertisement call.*

Darwin (*1*) proposed that sexual selection has two principal components: competition among males (intrasexual selection) and choice by females (intersexual selection). The role of male-male competition has been demonstrated (*2*); however, the importance of choice by females, especially choice based on male traits, has been disputed since Darwin first proposed the theory (*3*). Studies have shown that mate choice by females is based at least in part on resources controlled by males (*4*), and on male position on a lek (*5*), or rank in a social hierarchy (*6*). Attempts to show that females in natural populations prefer certain male morphological or behavioral characteristics have been frustrated by difficulties in separating the effect of these characteristics from that of the other factors influencing mate choice (*7*). Only laboratory studies of *Drosophila* have adequately demonstrated female choice based on male traits (*8*). I report that female choice of larger males influences male mating success in a neotropical frog, and that this choice is based on one male phenotypic characteristic, the fundamental frequency of an advertisement call component, which is correlated with size and probably age.

The breeding behavior of *Physalaemus pustulosus* (Leptodactylidae) was monitored in a small cement pool on Barro Colorado Island, Panama, for 12 weeks from June to August 1978. Individuals were captured, measured (snout to vent), and given a toe clip for permanent identification. Numbered pieces of surveyors' flagging were stitched to the middorsal surface, allowing undisturbed identification during behavioral observations.

Breeding in *P. pustulosus* occurs throughout the year, but is concentrated during the wet season (April to December). As with most anurans that have a prolonged breeding season, the sex ratio at the breeding site was skewed toward males (*9*). Males advertised from calling sites, and the females approached and initiated amplexus. Usually, a female was present in the pool only on the night she mated. The females seemed to choose their mates freely, although on several occasions noncalling males intercepted females that were en route to calling males. The males often fought each other, but in over 500 hours of observations during 1978 and 1000 hours in 1979, I never saw an unmated male displace a male in amplexus. Males constructed foam nests during amplexus by beating the jelly matrix of the egg mass with their hind legs as they fertilized the eggs (*10*). Nest building usually occurred 1 to 4 hours after the beginning of amplexus. The males were not territorial and did not defend resources. There was no relation between a male's calling site and the site used for oviposition or his ability to attract mates.

In 1978, I marked 185 males and observed 103 matings. As Fig. 1 shows, the larger males were more likely to acquire mates. However, only some of the males

0036-8075/80/0725-0523$00.50/0