

a major role in pain perception and eating behaviour. These factors may well contribute to the conflicting results that have been obtained in tests that rely on the suppression of behaviour by conditioned fear of a specific painful punishment and disruptive factors such as electric shock, food and water deprivation. In addition, the situation may well be complicated by the presence of functionally distinct 5-HT pathways which may be involved in the control of anxiety. Furthermore, the general lack of selectivity of the currently available drugs inevitably complicates any mechanistic interpretation and probably often contributes to a weakening of the effect when the selectivity involves diametrically opposed effects (e.g. pre- or postsynaptic agonist or antagonist). Thus the behavioural actions of drugs acting on the 5-HT system may be very dependent on the exact test situation studied.

Finally, although the data show that drugs which act on the 5-HT system can have significant effects in animal models of anxiety, the significance of these effects in predicting an effect on anxiety states in humans remains, in most cases, to be demonstrated. Of the drugs discussed above only buspirone is marketed as an anxiolytic, its anxiolytic activity having been observed clinically before it was 'confirmed' by animal tests. Other compounds such as ritanserin and ipsapirone have been shown in preliminary clinical testing to show anxiolytic potential. One might have expected that if the results of the animal tests presented in Table I are predictive of an action in humans, anxiolytic or anxiogenic activities would have been reported for at least some of these drugs that are used clinically. An anxiolytic activity, especially if it is not accompanied by sedation is not, however, the kind of side-effect of which patients readily complain. As to the anxiogenic effects, most of the drugs for which one might predict an anxiogenic effect are prescribed as antidepressants (either experimentally or very recently introduced) and very widely co-administered with a benzodiazepine!

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● Next month Jörg Traber and Thomas Glaser describe the detailed pharmacology of the 5HT_{1A} receptor-related anti-anxiety drugs buspirone, gepirone and ipsapirone in an article entitled: 5HT_{1A} receptor-related anxiolytics.

A new approach for evaluating the behavioral effects of antipsychotic drugs

Gene M. Heyman and Bernard Beer

One of the most researched topics in psychopharmacology is the effects of antipsychotic drugs on reinforced behavior. The basic finding is that antipsychotics decrease the frequency of reinforced behaviors at doses that do not have obvious effects on motor capacity. One interpretation is that the drugs acted on reinforcement processes. However, others contend that the decreases in reinforced behavior were due to subtle deficits in motor capacity. Gene Heyman and Bernard Beer introduce a quantitative procedure for dissociating changes in reinforcement efficacy and motor capacity. Experiments analyzed with this procedure showed that chlorpromazine and pimozide decreased reinforcement efficacy at doses that did not affect motor capacity.

In 1952 two French physicians, Delay and Deniker¹, announced that they had discovered an effec-

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tive pharmacological treatment for schizophrenia. They showed that an experimental drug, 4560 RP, reduced the frequency of hallucinations, bizarre speech, and agitation in hospitalized schizophrenic patients. These results suggested that drug therapies could reduce

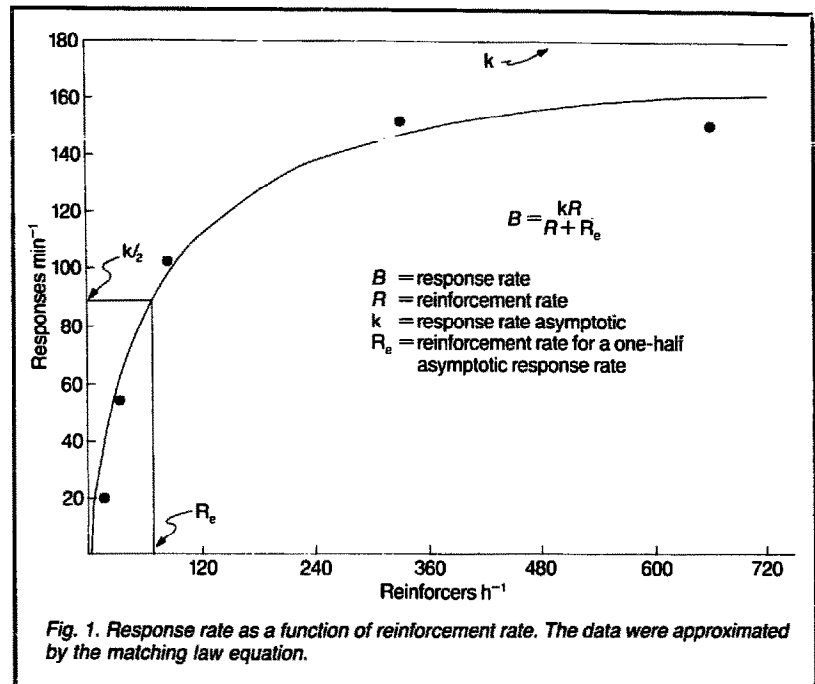
or even eliminate hospitalization for schizophrenia. Other clinicians replicated Delay and Deniker's results, and the drug, now known as chlorpromazine, rapidly became the treatment of choice for schizophrenia².

Following the introduction of chlorpromazine, other antipsychotic drugs were identified. In addition to their antipsychotic action, these drugs, often referred to as 'neuroleptics', shared a common behavioral effect. They attenuated reinforced (learned) behavior. For example, in the first published animal studies (1953), Courvoisier³ showed that chlorpromazine decreased the frequency of a learned response (climbing a pole to avoid shock) at doses that left reflexive movements intact. This finding suggested that neuroleptics might interfere with reinforcement (the process that maintains the learned response, see discussion below). However, others pointed out that chlorpromazine may have simply slowed motor performance, and that the effect was more apparent in relatively complex behaviors, such as pole climbing⁴. These interpretations spurred numerous studies, but despite a wealth of empirical findings, researchers still do not agree on the mechanisms mediating neuroleptic-induced changes in reinforced behavior⁵.

Importantly, the issue has clinical as well as theoretical implications. Firstly, among neuroleptics there is a strong correlation between the dose that attenuates reinforced responding and the dose used to treat schizophrenic patients⁴. Secondly, it is not clear if the clinical benefits of neuroleptics depend on the motor changes that these drugs can produce⁶.

In this article we describe an approach that we believe provides researchers with the tools for distinguishing between a change in reinforcement efficacy and a change in motor performance. The approach is based on Herrnstein's matching law equation⁷. However, before introducing this equation, something more needs to be said about reinforced behavior and antipsychotic drugs.

Learned behavior is maintained by its consequences. This can be demonstrated most simply with the traditional laboratory model of learning: a rat that has been

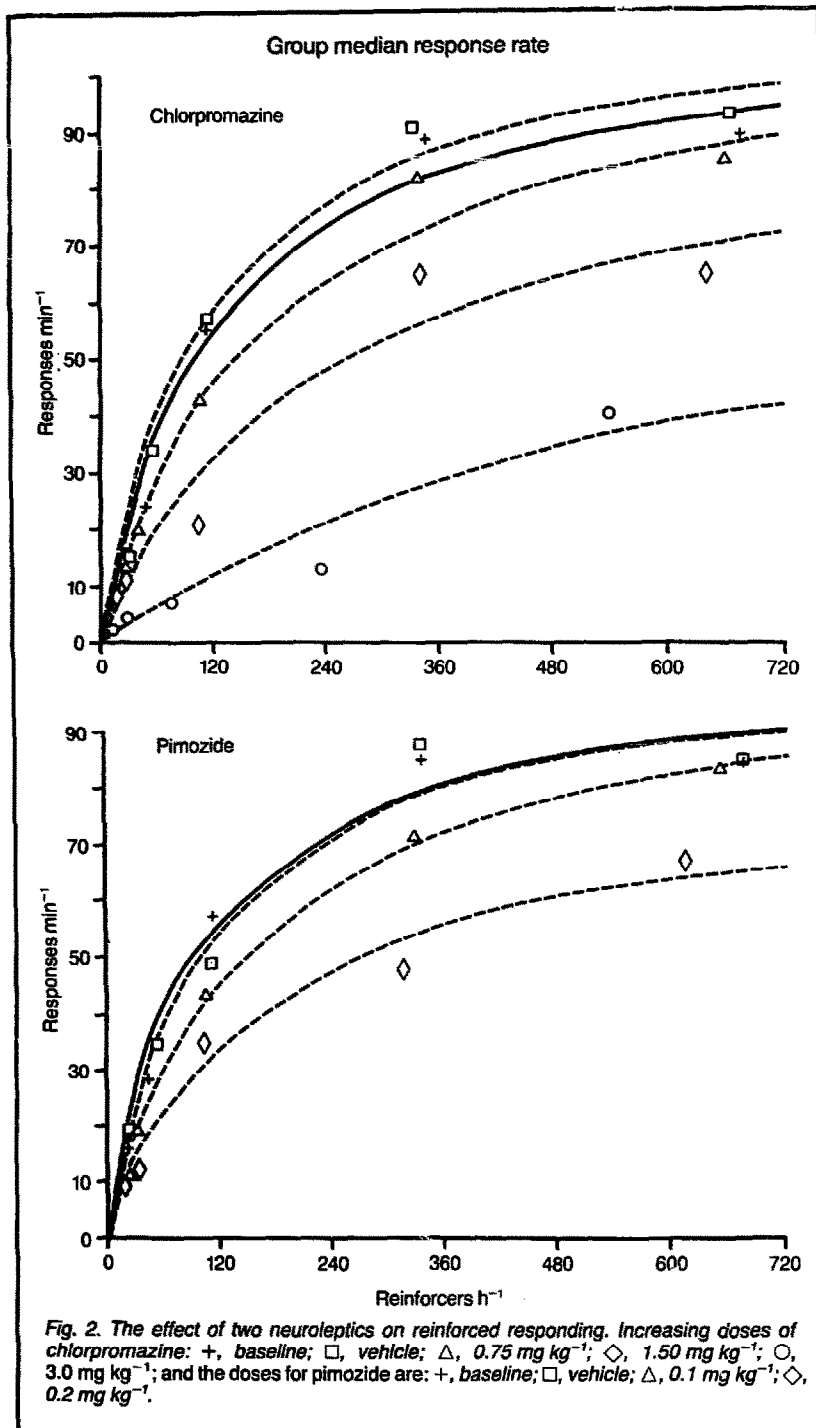


trained to press a lever. If the rat's food supply is limited then the frequency of lever pressing will increase if the response produces an increase in food intake. In contrast, the response will be extinguished if it no longer produces food. Dependent on deprivation state and species, events other than food can alter the frequency of learned behavior, and the longer the list of such events for a species, the wider the range of environments it can adapt to. Psychologists use the term 'reinforcer' to refer to events that affect the frequency of learning and the term 'reinforcement' to refer to the process that increases (strengthens) learned behaviors. Because of the importance of learning, psychopharmacologists have hoped to identify drugs that affect reinforcement. However, it is difficult to determine whether there has been a change in reinforcement efficacy, because the index, a change in the frequency of reinforced behavior, is dependent on several factors, including motor capacity, sensory processes, attention, and degree of deprivation. Fortunately, neuroleptics have been extensively researched, and the results allow us to simplify the problem: (1) hunger and thirst may be eliminated as factors, because neuroleptics did not affect water and food intake at dose levels that decreased water- and food-reinforced behavior⁸, and neuro-

leptics decreased responses that were maintained by reinforcers that are not consumable, such as brain stimulation⁸ and money⁹; (2) neuroleptic deficits in learned behaviors appear to be independent of sensory and attentional factors⁵, because increasing the intensity of stimuli that signal reinforcement did not improve performance⁴; but (3) in contrast, a correlation between changes in response rate and changes in response topography has been reported. Moderate doses of pimozide (a neuroleptic) decreased response rate and increased the duration of the response (slowed responding)¹⁰. However, in this experiment, the researchers evaluated only one possible mediating factor (response topography), and the data suggested that pimozide's effects were not exclusively motoric.

What is needed is a procedure that allows researchers to parse response rate. Ideally the method would yield independent, quantitative measures of the major factors that contribute to response rate. The matching law approach, as we show next, goes some way toward meeting these goals.

The matching law equation describes the quantitative relationship between reinforcement rate and response rate. Figure 1 shows some typical results along with the equation¹¹. The data are from a study in which water-deprived rats were trained to



press a lever, and the reinforcer, water, was varied over a wide range - about 20 to 700 drinks per hour. On the y-axis is response rate, lever presses per minute, and on the x-axis is reinforcement rate. The smooth line was obtained by fitting the equation to the data. The usual notation is:

$$B = \frac{kR}{R + R_e} \quad (1)$$

where B is response rate, R is

reinforcement rate, and k and R_e are fitted parameters. By definition, k is equal in magnitude to the response rate asymptote and R_e is equal to the rate of reinforcement that maintains a one-half asymptotic response rate. Importantly the results shown in Fig. 1 are typical of a much larger set. Similar quantitative findings have been obtained with different subjects (pigeons, monkeys, and humans) with different reinforcers (food, brain stimulation,

and money) with different response requirements (swimming, running, and pressing a telegraph key¹²). There is also a between-discipline generality - readers may recognize that the matching law is a rectangular hyperbola and that equations of this form describe quantitative aspects of enzyme kinetics and the relationship between drug dose and physiological response. These similarities will be returned to later.

Fig. 2 shows the effects of chlorpromazine and pimozide on the relationship between response rate and reinforcement rate in a study in which the matching law fit the data¹³. The subjects were water-deprived rats and the reinforcer was a small portion of water. The purpose of the study was to see how the neuroleptics would affect the parameters of the matching law equation. Low doses of chlorpromazine and pimozide decreased response rates maintained by the low reinforcement rates but not those maintained by the high reinforcement rates. This pattern of response-rate change produced an increase in R_e without altering k (see Ref. 13 for details and statistics). In contrast, higher doses suppressed responding maintained by both the low and high reinforcement rates. This pattern of changes led to changes in both k and R_e .

Fig. 3 shows the same results, but graphed in terms of a transformation of Eqn 1 that more readily reveals the changes in k and R_e . On the y-axis is the ratio of response rate to reinforcement rate and on the x-axis is response rate; the slope reflects the magnitude of R_e and the x-axis coordinate of the fitted line at $y = 0.0$ is equal to k . In other words, Fig. 3 is a Scatchard plot¹⁴, with response rate substituting for fraction of bound receptors and reinforcement rate for ligand concentration. This method of plotting the results shows that low doses of pimozide and chlorpromazine increased the magnitude of R_e and that higher doses reduced the value of k and produced further increases in R_e . Thus, these two neuroleptics increased the rate of reinforcement necessary for a one-half asymptotic response rate and also reduced the asymptotic response rate.

Studies conducted in other laboratories have also shown that pimozide changed both k and R_e ¹⁵⁻¹⁷. Moreover, in these studies the reinforcer was brain stimulation^{16,17} and food¹⁵ (in Figs 2 and 3 the reinforcer was water). However, there is an apparent exception. Morley, Bradshaw and Szabadi¹⁸ obtained results that suggested that pimozide decreased k without altering R_e . However, they inferred values of k and R_e , because their experiment did not supply enough data points to estimate these parameters (there were only two different reinforcement schedules), and they did not empirically check if their procedure produced results similar to those in which there was a sufficient number of data points to fit a two parameter equation. Thus, it is not possible to tell if these data are discrepant or simply reflect measures that do not correspond to k and R_e .

The results in Figs 2 and 3 are orderly, but require interpretation. Our approach is twofold. First on the basis of a recent literature review¹¹, we summarize the results from experiments in which k and R_e were the dependent variables. For example, in a number of studies, the experimenter altered the response requirement and measured changes in k and R_e ^{11,19}. Second, we derive definitions of k and R_e on the basis of elementary properties of reinforced behavior (see Box). We found nine studies in which the experimental manipulation systematically altered R_e but did not produce any apparent change in k ¹¹. In each of these studies the experimental manipulation was either a change in deprivation or a change in some property of the reinforcer, such as magnitude. For example, in rats, changing the reward from glucose to sucrose increased low response rates selectively so that R_e^* decreased by about 36% without any change in k (Ref. 12). Similarly, in another study with rats in which water was the reinforcer, varying the deprivation period from 6.5 to 47.5 hours systematically decreased R_e without any apparent change in k (Ref. 11). Deprivation, reward magnitude, and reward quality affect the capacity of a reinforcer to maintain behavior. Thus, factors

*The smaller the value of R_e the greater the response rate for a given reinforcement rate.

that affect reinforcement efficacy affect R_e .

We found four studies in which the manipulation systematically affected k without affecting R_e (Ref. 11). In each of these experiments, the manipulation was a change in the response requirement. For example, in a study with pigeons, changing the response requirement from a key-peck to a treadle-kick brought

about large changes in k with no apparent effect on R_e (Ref. 19). Changes in the response requirement necessarily alter the topography of the response, for example its duration. Thus factors that affect response topography affect k .

There are also several studies in which changes in the properties of the reinforcer or deprivation altered k as well as R_e (Ref. 11).

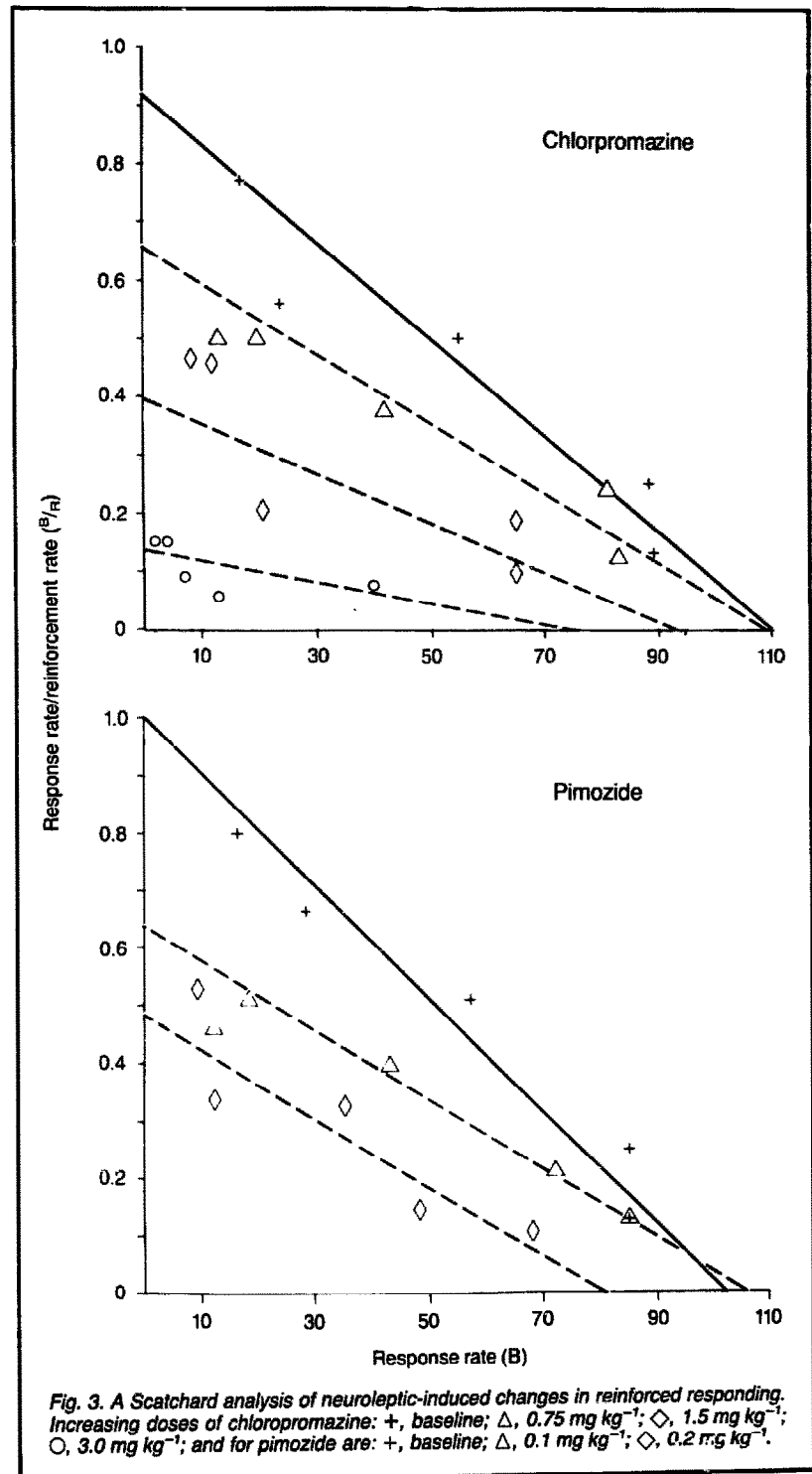


Fig. 3. A Scatchard analysis of neuroleptic-induced changes in reinforced responding. Increasing doses of chlorpromazine: +, baseline; Δ , 0.75 mg kg⁻¹; \diamond , 1.5 mg kg⁻¹; \circ , 3.0 mg kg⁻¹; and for pimozide are: +, baseline; Δ , 0.1 mg kg⁻¹; \diamond , 0.2 mg kg⁻¹.

Derivation of the matching law

It is possible to show that the matching law is a logical consequence of certain basic features of operant experiments²². The critical features include the following: (1) The environment contains a manipulandum that provides access to reinforcement; (2) reinforcement occurs intermittently (and unpredictably); (3) the subject switches back and forth between the reinforced activity and other alternative activities, such as resting, exploring, etc. The simplest possible rule for switching into the reinforced activity is that it is proportional to the reinforcement rate and available time:

$$S_1 = v_1(T_s - T_r)R \quad (A1)$$

where S_1 is the overall switching rate into reinforced activity, T_s is the session duration, T_r is the total amount of time spent in the reinforced activity, and v_1 is the switching constant. Similarly, the rule for switching out of reinforced activity into alternative activity is:

$$S_2 = v_2 T_r \quad (A2)$$

where v_2 is the constant for switches in this direction. When the proportions of time spent in reinforced and alternative activities are stable, it must be the case that the two overall switching rates are the same, that is $S_1 = S_2$. Thus for a stable or equilibrium state we can set Eqn A1 equal to Eqn A2 and solve for the relationship between reinforcement rate and time spent in the

reinforced activity. After simple rearrangement the result is:

$$T_r = \frac{T_s R}{R + v_2/v_1} \quad (A3)$$

Eqn A3 like the matching law is a rectangular hyperbola. However, Eqn A3 relates time spent responding to reinforcement rate and the matching law relates response rate to reinforcement rate. For environments in which the subject responds at a constant tempo while it is engaged in reinforced activity, as is usually the case²², Eqn A3 can be rewritten.

$$B = \frac{MR}{R + v_2/v_1} \quad (A4)$$

where M is response tempo, e.g. two response sec^{-1} . Eqn A4 says that the response rate asymptote is determined by the tempo of responding and the rate of reinforcement that maintains a one-half asymptotic response rate is determined by the likelihood of switching in and out of reinforced activity. These logically deduced definitions agree with the data. For example, switching rates should depend on reinforcement factors, and as outlined above there is a correlation between changes in R_e (v_2/v_1) and changes in either deprivation or reward parameters.

These results conflict with the nine experiments in which only R_e changed. However, there is evidence that the discrepant findings were due to methodological factors (see Ref. 11 for discussion). Thus we conclude that the simplest account of the results is that k is a measure of the topography of the reinforced response (its duration) and that R_e is a measure of the efficacy of the reinforcer maintaining the response. Thus according to the matching law criteria, low doses of pimozide and chlorpromazine decreased reinforcement efficacy, whereas the higher doses slowed the tempo of responding and also produced further decreases in reinforcement efficacy. In other words, by the standards that we are advocating, psychopharmacologists have accomplished an important goal - identifying drugs that affect reinforcement processes.

The matching law equation has the same form as the Michaelis-Menten equation for enzyme kinetics²⁰ and the equation A. J. Clark²¹ derived for describing the relationship between drug dose and physiological response. In the case of enzyme kinetics and drug response, the mathematical models reflect an equilibrium state between opposing processes, for

example the tendency for drug molecules and receptors to bind and dissociate. An analogous equilibrium state can be identified in reinforced behavior experiments. The subject is either engaged in the reinforced task, for example, pressing a lever, or alternative behavior, like exploring the chamber, resting, etc. When response rates are stable, the proportion of time spent in these two states are necessarily in equilibrium. Elsewhere, it is shown that the matching law is a logical consequence of equilibrium between time spent in the reinforced activity and time spent in alternative activity²². Interestingly, the derivation yields the interpretation that R_e is a measure of reinforcement efficacy and that k is a measure of response topography. Thus theory and the empirical data independently converged to the same definitions of k and R_e .



In schizophrenics, neuroleptics reduce agitation, flatten affect, and decrease the number of reported delusions and hallucinations. The drugs also produce parkinsonian motor deficits. Estimates of how many patients experience tremor or rigidity vary,

but some experts report that mild motor dysfunctions occur in all those effectively treated with neuroleptics⁶ and moderate to severe deficits occur in as many as 60% (Ref. 23). It is possible that the clinical and laboratory neuroleptic syndromes share much in common. The parkinsonian symptoms in patients and the changes in k of the matching law equation in rats may reflect the same sort of motor deficit. The neuroleptic-induced changes in cognition may be secondary to changes in simple reinforcement processes or even activity level. These links have yet to be explored, but seem to us lines of inquiry that could lead to more effective pharmacological treatments for schizophrenia and a better understanding of this debilitating disease.

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Regulation of the L-type calcium channel

Franz Hofmann, Wolfgang Nastainczyk, Axel Röhrkasten, Toni Schneider and Manfred Sieber

The voltage-operated L-type calcium channel is regulated by protein phosphorylation and G proteins in a variety of tissues and eukaryotes including non-excitabile cells. The 165 kDa protein of the dihydropyridine receptor from rabbit skeletal muscle contains all the regulatory sites of an L-type calcium channel and the calcium conducting unit. Franz Hofmann and colleagues suggest that the differences in the regulation observed in various tissues is caused by the interaction of the large conducting protein with different regulatory proteins of approximately 55 kDa.

There is hardly a tissue where calcium channels have not been postulated as an important part of the signal transduction mechanism. They have emerged early in evolution and have been found throughout eukaryotes including protozoa, algae, higher plants, fungi and animals. Voltage-operated calcium channels are a key component of all excitable cells which transduce electrical signals into biochemical events. In contrast, receptor-operated calcium channels have not been detected by electrophysiological techniques to date, but recent evidence suggests that excitable and non-excitabile cells have calcium channels which are regulated indirectly by hormone receptors.

Hormonal regulation of calcium channels

Voltage-operated calcium chan-

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nels have been differentiated into T-(transient), N-(neuronal) and L-(long lasting) channels (see Box). N-channels are apparently present only in neuronal cells whereas T- and L-channels have been identified in most cells tested. The cardiac L-type calcium channel was the first channel known to be modulated by hormones¹⁻³ (see also Table I). Stimulation of ventricular β_1 -adrenergic receptors activates cAMP-dependent protein kinase and increases 3 to 4-fold voltage-dependent calcium influx.

Perfusion of isolated myocytes with a variety of compounds and enzymes shows that cAMP kinase phosphorylates L-channels or a protein closely associated with L-channels. Single channel recording suggests that phosphorylation decreases the closed times, increases the open time and decreases about 3-fold the number of blanks, i.e. tracings in which the channel does not open upon depolarization. These changes increase about 4-fold the probability that the channel is open and is available for voltage-dependent opening. About 20%

of the cardiac L-channels open in the absence of cAMP-stimulated phosphorylation. Channel opening cannot be decreased further by perfusion of a single myocyte with the specific kinase inhibitor protein, a large excess of the regulatory subunit of cAMP kinase⁴ or the catalytic active fragment of protein phosphatase I (Ref. 5). This suggests that cAMP-dependent phosphorylation is not a prerequisite for voltage-dependent channel opening.

In contrast, an absolute dependence of channel opening on cAMP-dependent phosphorylation has been observed in an isolated patch of a L-channel excised from a neurosecretory cell line⁶. Unlike neuronal L-channels, no mechanism has been identified in cardiac ventricular cells which modulates L-channels without affecting the activity of cAMP-dependent protein kinase.

Phosphorylation of the cardiac L-channel is stimulated *in vivo* by all hormones which activate adenylate cyclase. An increased current can be decreased by hormones which inhibit adenylate cyclase activity. Stimulation of ventricular muscarinic receptors decreases the calcium current through the latter mechanism by activation of the inhibitory GTP-binding protein G_i . The calcium current is also decreased by cGMP which lowers the cAMP level by activation of a cGMP-stimulated cAMP phosphodiesterase⁷. Both mechanisms result in a dephosphorylation of the channel and thereby decrease the open state probability.

Neuronal calcium currents are inhibited by a variety of adrenergic and peptidergic receptors (see Table I). These receptors activate a pertussis toxin-sensitive GTP-binding protein, which couples