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CELLULAR ANALOGS OF OPERANT BEHAVIOR

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13. ABSTRACT (Maximum 200 words) Our previous work indicates that hippocampal CA1 bursting may be reinforced by dopaminergic agents such as dopamine itself, cocaine, and certain dopamine receptor agonists. A major concern is that these agents may facilitate bursting merely by direct or indirect pharmacological stimulation of neuronal activity rather than by a cellular reinforcement process. We have always required as critical evidence of cellular reinforcement that noncontingent or random presentations of the positive agents will be relatively ineffective; and indeed random applications of dopamine, cocaine, and dynorphin A are ineffective and even tend to suppress the bursting of hippocampal pyramidal cells. One approach is to attempt to reinforce hippocampal bursting with a nonspecific depolarizing agent such as glutamate. Unlike dopamine and cocaine, burst-contingent applications of glutamate did not produce selective facilitation of cellular bursting when compared to random presentations; indeed, both contingent and random glutamate applications reduced the likelihood of bursts, while at the same time increasing the frequency of individual spikes. These results are consistent with the idea that dopamine's reinforcing action on hippocampal bursting cannot be attributed to nonspecific stimulation. The burst-suppressant action of glutamate is intriguing, and suggests that glutamate mechanisms might normally function in opposition to the dopamine reinforcement mechanisms.

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Good progress has been made in the Dec 89-Nov 90 period of our project "Cellular Analogs of Operant Behavior". The principal aims are to a) demonstrate the operant conditioning of individual cellular activity in an in vitro brain slice preparation and b) to determine whether or not such cellular operant conditioning underlies behavioral operant conditioning in vivo. If the two types of operant conditioning are interrelated, they should exhibit common properties. Thus, it might be possible to show that both forms of operant conditioning utilize the same reinforcing transmitters and drugs and that the same receptor subtypes mediate these reinforcing effects.

The work is organized in two main parts - cellular operant conditioning studies and behavioral operant conditioning studies.

I. Cellular Operant Conditioning

A. Opposite Effects of Dopamine and Glutamate on Hippocampal CA1 Operant Conditioning. (Xue, B.G. and Stein, L., *Soc. for Neurosci. Abstracts*, 16:261,1990.)

Our previous work indicates that hippocampal CA1 bursting may be reinforced by dopaminergic agents such as dopamine itself, cocaine, and certain dopamine receptor agonists. A major concern is that these agents may facilitate bursting merely by direct or indirect pharmacological stimulation of neuronal activity rather than by a cellular reinforcement process. We have always required as critical evidence of cellular reinforcement that noncontingent or random presentations of the positive agents will be relatively ineffective; and indeed random applications of dopamine, cocaine, and dynorphin A are ineffective and even tend to suppress the bursting of hippocampal pyramidal cells. One approach is to attempt to reinforce hippocampal bursting with a nonspecific depolarizing agent such as glutamate. Unlike dopamine and cocaine, burst-contingent applications of glutamate did not produce selective facilitation of cellular bursting when compared to random presentations; indeed, both contingent and random glutamate applications reduced the likelihood of bursts, while at the same time increasing the frequency of individual spikes. These results are consistent with the idea that dopamine's reinforcing action on hippocampal bursting cannot be attributed to nonspecific stimulation. The burst-suppressant action of glutamate is intriguing, and suggests that glutamate mechanisms might normally function in opposition to the dopamine reinforcement mechanisms.

B. Reinforcement of Hippocampal CA1 Bursting by Cannabinoid Receptor Activation. (Xue, B.G. and Stein, L., *Soc. for Neurosci. Abstracts*, in press.)

Involvement of cannabinoid receptors in behavioral reinforcement has been demonstrated in animals by self-administration of Δ^9 -tetrahydrocannabinol (THC) and in humans by the addictive properties of marijuana and related agents. Furthermore, cannabinoid receptors and reinforcement-relevant dopamine D2 and μ -opioid receptors are known to share the same signal transduction mechanisms and

have in common the ability to activate G_i proteins that inhibit adenylate cyclase. Accordingly, it was of particular interest to determine whether or not cellular operant conditioning could be demonstrated with cannabinoid receptor activation as reinforcement. The high affinity cannabinoid agonist CP-55940 was used as the reinforcer for CA1 hippocampal operant conditioning (cannabinoid receptors are present in high density in rat hippocampus). Highly reliable CA1 operant conditioning was obtained; more than 55% of the neurons tested were successfully reinforced by burst-contingent applications of CP-55940 (at concentrations of 5 and 10 μ M, but not at 2.5 or 100 μ M). The same microinjections, administered independently of firing, did not increase bursting rate and therefore provided a control for direct pharmacological stimulation of cellular activity. Co-administration of forskolin (which activates cyclic AMP formation) eliminated the reinforcing action of CP-55940, consistent with the idea that cannabinoid reinforcement may involve inhibition of cyclic AMP formation. The results indicate that cannabinoid receptor activation can reinforce hippocampal CA1 bursting and suggest that cannabinoid receptors, like dopamine and opioid receptors, may play important roles both in behavioral and cellular operant conditioning.

C. Dopamine Receptor Subtype and Cellular Reinforcement

Five dopamine receptors are presently recognized, which may be divided on the basis of homology and pharmacological similarity into two main dopamine receptor subgroups, D₁-like (D₁ and D₅) and D₂-like (D₂, D₃, D₄). In early experiments, we showed that the D₂-preferring agonist N-0437 was an effective reinforcer of hippocampal CA1 bursting activity, whereas the D₁ agonist SKF38393 was ineffective. To establish the specificity of N-0437's action at D₂ receptors, we compared the activity of its optical isomers, N-0923 and N-0924, which differ by 100-fold in D₂ potency. In the dose range 1-6 mM, only the D₂-active isomer N-0923 was effective as a reinforcer of CA1 bursting; even at the highest concentration of 6 mM, N-0924 was inactive. Most recently, we have been conducting CA1 operant conditioning using quinpirole, a D₃-preferring agonist, as reinforcement. Whereas the D₂ receptor is found in the majority of tissues innervated with dopamine, D₃ receptors are present in high densities only in motivationally-relevant limbic forebrain areas. To our surprise, quinpirole was active as a reinforcer at 0.025 mM--approximately 20 times more potent than dopamine itself. Quinpirole has 5 times greater affinity than dopamine for the D₃ receptor, and it also is more resistant to degradation--hence, the 20-fold potency differential is consistent with the possibility that the D₃ receptor subtype plays a major role in the mediation of reinforcement.

II. Behavioral Operant Conditioning

A. Mu-receptor mediation of dynorphin self-administration (Stevens, K.E., Shiotsu, G. and Stein, L., *Brain Res.*, 545:8-16, 1991.)

Drug-naive rats rapidly learned to self-administer dynorphin A in the CA3 region of hippocampus, confirming prediction from our earlier cellular work in which dynorphin A was found to reinforce the bursting activity of CA3 neurons. In subsequent behavioral studies, dynorphin A self-administration was blocked by co-administration of the opioid antagonist naloxone, which indicated that dynorphin's reinforcing effects are exerted at an opioid receptor. To establish which opioid receptor subtype may mediate dynorphin self-administration, co-administration studies were carried out with highly selective mu, delta or kappa antagonists. Only

the selective mu-antagonist β -funaltrexamine blocked dynorphin self-administration. It was concluded that mu receptors in the CA3 region of hippocampus are important target sites for dynorphin reinforcement.

B. Dopamine Receptor Subtypes in Behavioral Reinforcement. (Self, D.W. and Stein, L., *Soc. for Neurosci. Abstracts*, in press.)

The reinforcing properties of the selective D₂ agonists N-0437 and N-0923 were demonstrated for the first time in our cellular operant conditioning experiments. If cellular and behavioral reinforcement mechanisms are interrelated, N-0923 should also serve as an effective reinforcer of behavior. Rats were trained in daily 3-hour sessions to intravenously self-administer cocaine (0.75 mg/kg/injection) by pressing a bar. A second bar delivered no injections and provided a control for nonspecific stimulation. After cocaine self-administration had stabilized, various doses of N-0923 or d-amphetamine were substituted for the cocaine reinforcement. N-0923 was avidly self-administered, and in fact was substantially more potent than either amphetamine or cocaine. In a second experiment, we attempted to determine the relative contribution of D₁ and D₂ receptor activation to the reinforcing action of cocaine. Cocaine self-administering rats were pretreated before the test session with either the D₁ agonist SKF 38393 or the D₂ agonist N-0923. It is well established that if a self-administering rat is pretreated with a reinforcement enhancer (such as cocaine itself), the average interval between successive self-administrations is increased and the self-administration rate is decreased; on the other hand, if the pretreatment drug blocks reinforcement, the inter-injection interval is shortened and the self-administration rate is increased. Cocaine self-injections were decreased in a dose-dependent manner by the D₂ agonist N-0923 and increased in a dose-dependent manner by the D₁ agonist SKF 38393. The results support the idea that D₂, but not D₁, receptor activation facilitates the reinforcing action of cocaine.

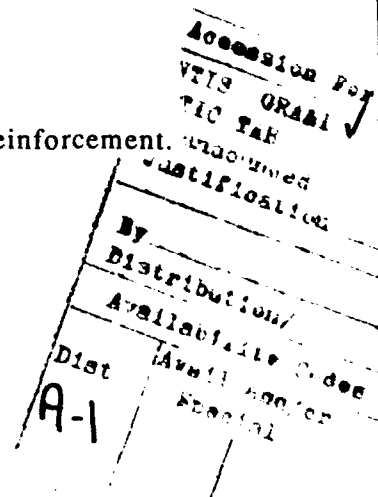
Work initiated in a previous grant period on the role of endogenous opioids in reinforcement function was analyzed and published this year (Trujillo, K.A., Belluzzi, J.D., and Stein, L., *Psychopharmacology*, 104:265-274, 1991.) This work showed that very low doses of naloxone, without effect when tested by themselves, can block the reinforcing effects of amphetamine in conditioned place preference. These results provide evidence of interactions between endogenous opioids and catecholamines in the mediation of reinforcement processes.

III. Project Personnel

Dr. B. Xue has performed the technically demanding electrophysiological experiments, with great skill. David Self, a senior graduate student, has replaced Dr. E. Sehitoglu as supervisor of the behavioral work. Drs. McAfee and Belluzzi have separate support and Mr. Self is almost entirely supported by a PHS fellowship.

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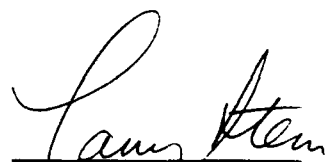
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