*Proc. Indian natn Sci Acad.(PINSA)* **B67** Nos 3 & 4 pp155-188 (2001)

# Intracranial Self - Stimulation: An Animal Model to Study Neuronal Plasticity, Drug Addiction and Depression - A Review

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(Received on 2 November 2000; after revision on 13 June 2001; Accepted on 12 August 2001)

Rewards can exert a powerful control over our thoughts and our behavior. It is assumed that the mechanism of these effects is in the brain, and neurobiologists have been tempted by the possibility that there is as much to be learned about reward from the study of its mechanism as from the study of its behavioral phenomenology. James Olds and Peter Milner in 1954 at McGill University, made an intriguing but puzzling discovery by accident and demonstrated that a rat with electrodes inserted into certain areas of its brain would learn an arbitrary response like pressing a pedal to obtain electrical stimulation of the brain itself and this phenomenon is known as self-stimulation (SS) or intracranial self-stimulation (ICSS). It has been subsequently shown that ICSS is not an idiosyncrasy of the rat, but can be obtained from many vertebrates, including humans. Animals will work for a reward, which is in the form of electrical stimulation at certain specific sites within the brain and James Olds termed these as 'pleasure centers'. Electrical self-stimulation behavior has been considered as one of the intensely rewarding behavioral experiences, perhaps even more influential than feeding or sexual rewards. The most significant contribution of this discovery in the behavioral neuroscience is that, it provided a window to peep into the brain to identify the neural substrate of reinforcement, which is a critical requirement for higher mental functions such as learning and memory. It is an outstanding experimental model, which can be used to relate the dynamic and plastic properties of behavior to a direct manipulation of the brain activity. ICSS also provides an excellent animal model to explore the neural substrate responsible for drug addiction. The present paper is a review of evidence implicating the ICSS as a model for drug addiction, depression and neuronal plasticity.

Key words: **Depression, Dopamine transporters, Drug addiction, Intracranial self-stimulation, Mesolimbic dopamine system, Mood disorders, Motivation, Reinforcement, Neuronal plasticity, Psychostimulants, Reward centers, Self-administration**

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**Abbreviations:** ACh, acetylcholine; AChE, acetylcholinesterase; BSR, brain stimulation reward; CCK, cholecystokinin; CNS, central nervous system; CPU, caudate putamen; CRF, continuous reinforcement; DA, dopamine; DAT, dopamine transporter; DβH, dopamine β-hydroxylase; DNAB, dorsal noradrenergic bundle; DOPAC, 3,4-dihydroxy phenyl acetic acid; EAA, excitatory aminoacid; fMRI, functional magnetic resonance imaging; GABA, g-Aminobutyric acid; 5-HIAA, 5-hydroxyindole acetic acid; 5-HT, 5-Hydroxytryptamine; ICSS, intracranial self-stimulation; LC, locus coeruleus; LH, lateral hypothalamus; LH-MFB, lateral hypothalamus-medial forebrain bundle; LTP, Long-term potentiation; MAO, monoamine oxidase; MFB, medial forebrain bundle; NA, noradrenaline; nACB, nucleus accumbens; NE, norepinephrine; NMDA, N-methyl-D-aspartate; NT, neurotensin; OT, olfactory tubercle; 6-OHDA, 6-Hydroxydopamine; PCPA, para-chlrophenyl alanine; SN-VTA, substantia nigra-ventral tegmental area; SN, substantia nigra; SPE, stimulation produced energisation; SS, self-stimulation; VTA, ventral tegmental area.

## Introduction

Understanding the physiological basis of behavior is one of the major goals in the contemporary neuroscience. The modern approach is to view the brain as a collection of interconnected neural networks that control various aspects of behavior. James Olds and Peter Milner in 1954 at McGill University, Canada, made an intriguing but puzzling discovery by accident and demonstrated that a rat with electrodes inserted into certain areas of its brain would learn an arbitrary response like pressing a pedal to obtain electrical stimulation of the brain itself. Olds and Milner originally intended to investigate the effect of electrical stimulation of the reticular formation on the arousal and facilitation of learning process in the rats. Fortuitously, one of the electrodes missed its target and the tip reached a few millimeters away from the original target, probably in the septum/hypothalamus (Olds 1962). Further studies revealed that various other brain regions were also capable of producing the same effect and these centers were called 'pleasure centers of the brain' (Wise 1980b).

The original proposal of 'pleasure centers of the brain' was dropped for its philosophical connotation and it was attributed to a hypothetical reinforcement reward called brain stimulation reward (BSR) and the technical term intracranial self-stimulation (ICSS) or just self-stimulation (SS) was adopted. ICSS is supposed to represents a rewarding effect of the brain stimulation and the reward represent brain stimulation elicited feeling of pleasure in a hedonic sense. The presence of reinforcement is inferred from stimulation seeking behavior of the animal. The anatomical structures subserving ICSS have been variously designated as 'reward pathways' (Wise 1980a), 'reward circuitry' (Wise & Bozarth 1984) and 'reinforcement mechanisms' (Jenck et al. 1987, Wise & Rompre 1989).

The most significant contribution of this discovery in the behavioral neuroscience is that, it provided a window to peep into brain to identify the neural substrate of positive reinforcement and subjective aspects of behavior. Besides providing a new dimension to study the brain-behavior relationship, it is an outstanding experimental model and can be used to relate the dynamic and plastic properties of behavior to a direct manipulation of the brain activity. This discovery elicited a lot of publicity and it was reported that 'our pleasure centers' have been found, which can cure all types of problems such as depression and schizophrenia. The later is thought to be the result of actions of loosing their reward-anhedonia were just around the corner as depressed animals fail to enjoy rewarding stimuli a characteristic in both depression and schizophrenia. Recently, the electrical self-stimulation paradigm has proved to be very useful in delineating the neural substrate involved in learning, mental disorders and drug abuse. Accordingly, neural basis of brain stimulation reward and the studies related to the use of ICSS as a model to evaluate the neuronal plasticity, drug addiction and depression is reviewed below.

## Characteristics of ICSS

(i) Intense and persistent response rates of over 5000/hr when stimulated from lateral hypothalamus (LH) (Olds 1962) and 8000/hr from ventral tegmental area (VTA) (Phillips & Lepiane 1986) in rats and over 17,000/hr from LH in monkeys (Routtenberg et al. 1971) have been reported. This can occur for many hours until the animal is exhausted. At other sites, however, intermittent pauses begin to occur after an hour or so and become prolonged as time goes on. It can be speculated that the reward from these points is not so strong to overcome the muscular fatigue as a consequence of repeated pedal pressing or to compete with other motives like food or sex (for other details refer table 1).

(ii) The ICSS tasks involve extremely rapid rate of acquisition.

(iii) The responding strength depends on the stimulating current intensity, frequency and train duration, etc. Good rate-intensity and rate-frequency functions can be obtained (Campbell et al. 1985, Phillips et al. 1986).

(iv) Different types of operant and other tasks can be used like alley running (Edmonds & Gallistel, 1977), maze (Cazala 1986, Mason et al. 1985), and lever pressing (Shankaranarayana Rao 1992, 1996) for eliciting ICSS behavior.

(v) There is a preference for electrical reward over the conventional natural rewards like food, water and sex (Routtenberg 1978).

(vi) There is a lack of apparent satiation i.e., relatively unsatiable, as animals may continue lever pressing for long periods.

(vii) The behavior is remembered by the animal for several months and this indicates the permanent learning and memory.

(viii) Spontaneous extinction on continuous reinforcement (CRF) or intermittent reinforcement schedule can occur (Culbertson et al. 1966, Fouriezos & Wise 1976). If the reward is no longer given, the animal will almost immediately stop the behavior. This is very different from what occurs when rats or other animals are trained to press the lever for a natural reward like food or water (Howarth & Deutsch, 1982). In the latter case, animals will usually continue to press the lever for a longer time even after reinforcement has been discontinued.

(ix) Stimulation produces energisation (SPE) is variously termed as priming (Gallistel et al. 1981), incentive (Koob 1977) or forcement (Atrens 1984).

## How Can One Measure an Animals' Feeling of Pleasure?

The magnitude of response to electrical stimulation of the reward sites can be measured by means of Skinner's technique of operant conditioning. An animal with electrodes implanted at the appropriate sites is put in a 'do it yourself' situation, where it presses a lever to stimulate its own brain. One can translate the animals' strength of desire to get electrical stimulation into response frequency, which can be measured (Bindu 1990, Shankaranarayana Rao 1992, 1996, Singh 1990).

## Brain Areas that Produce ICSS

By systematically varying the position of the stimulating electrode, it is possible to map those regions of the brain from which ICSS can be obtained (table 1 & 2). Two features are immediately apparent, (i) ICSS can be obtained from sites distributed very widely in the brain, and (ii) the functions associated with the structures that support ICSS are very diverse. Thus, the ICSS can be obtained from areas in the brain that vary in location and in their function.

Eventhough several areas in the brain have been identified for ICSS, LH-MFB (Gratton & Wise 1983) and SN-VTA (Corbett & Wise 1980, Wise 1981) sites produce the strongest expression of reward. The average pedal press responses in 15 min sessions from left and right LH-MFB and SN-VTA regions are 1806 ± 170, 1879 ± 105 and 1952 ± 132, 2030 ± 116, respectively. Shankaranarayana Rao (1996) has mapped the placement of electrodes in LH-MFB and SN-VTA regions bilaterally in self-stimulation experienced rats as shown in figure 1. The electrode sites which produce strongest expression of ICSS were range from bregma –1.8 to –3.3 mm in LH-MFB and –4.8 to –6.3 mm in SN-VTA, which corresponds to the rat atlas plates of Paxinos and Watson (1982).

## Reward and Punishment

At about the same time of the discovery of brain electrical stimulation reward, Delgado and his colleagues (1954) showed that the activation of certain areas of the brain, on the contrary produced vigorous escape responses in cats and seemed to have strong aversive effects which prompted the animals to interrupt the stimulation, i.e., the animal will do some other act to avoid stimulation. These regions include periventricular area, the dorsal part of the mesencephalic central gray, medial thalamus, amygdala, hippocampus, hypothalamus including ventromedial area and various other regions involved in the regulation of nociceptive phenomenon i.e., the medial lemniscus and lateral and ventral reticular formation. Cazala (1986) could elicit ICSS from these aversive regions of the brain when the animals were placed in a maze where they could successfully trigger and turn off electrical stimula-

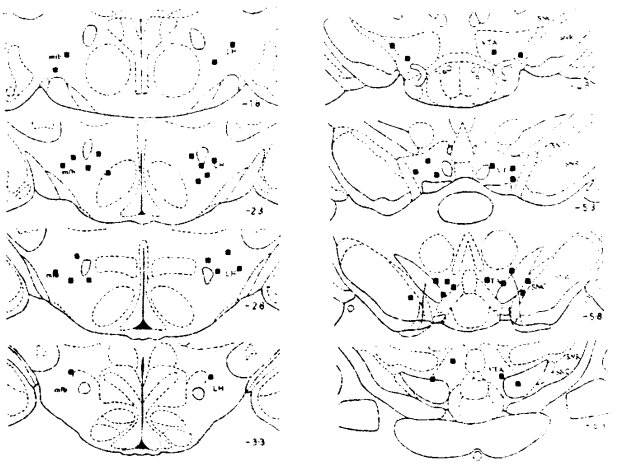

**Figure 1** Mapping of electrode placements in LH-MFB (A) and SN-VTA (B), billaterally in ICSS experienced (n=12) rats (Shankaranarayana Rao 1996).

**Table 1** *Positive and negative aspects of different brain regions in the ICSS production*

| Brain Region | Positive and Negative Aspects with regards to ICSS |
|---|---|
| Prefrontal and Entorhinal cortex | Slow acquisition, small target, but well characterized anatomically and pharamacologically |
| Septal area | Seizures, lack of rate-dependency |
| Nucleus accumbens | Slow acquisition, but no stimulation produced energization (SPE), seizures possible |
| Lateral hypothalamus | Fast acquisition, high percentage of positive animals, SPE possible, anatomical complexity, placements not associated with kindling of seizure activity, good rate-intensity function |
| Ventral tegmental area | Fast acquisition, minimum training time, seizure free, highest rate possible, good rate-intensity function |
| Substantia nigra | Forced movements, but not seizures, fast acquisition, minimum training time, good rate-intensity function |
| Periaqueductal gray | Possible ambivalence, rapid learning, good rate-intensity function |
| Hebenular nucleus | Small target, slow acquisition, good rate-intensity function |
| Subfornical organ | Small target, no priming |
| Fornix | Rapid acquisition |
| Cerebellum | Slow acquisition, forced movements, no SPE |
| Motor trigeminal nucleus | Forced oral movements |

(From Shankaranarayana Rao 1992, 1996)

**Table 2** *Brain regions associated with ICSS in the rat*

| | |
|---|---|
| **Telencephalon** | Prefrontal cortex, Entorhinal cortex, Olfactory bulb, caudate-putamen (striatum, globus pallidus), nucleus accumbens, peduncular nucleus, lateral septal area, hippocampus (dentate gyrus), amygdale |
| **Diencephalon** | Ventral and medial thalamus, lateral hypothalamus-medial forebrain bundle, anterior hypothalamus, ventromedial hypothalamus, substantia innominata, Habenular nucleus |
| **Mesencephalon** | Ventral tegmental area (A10), substantia nigra (A9), Dorsal and medial raphe, dorsal reticular formation, superior cerebral peduncle, periaqueductal gray, mesencephalic nucleus of trigeminal organ, interpeduncular nucleus, subfornical organ, fornix |
| **Metencephalon** | Locus coeruleus (A6), Dorsal noradrenergic bundle, cerebellum, deep cerebellar nuclei (dentate nucleus), cerebellar peduncle, motor trigeminal nucleus |
| **Myelencephalion** | Nucleus tractus solitorius |

(From Shankaranarayana Rao 1992, 1996)

tion. Miserendino and Coons (1989) demonstrated 'pure reward' and 'reward - escape' behaviors by electrical stimulation of medial prefrontal cortex. The anatomical and pharmacological studies of aversion mediating systems have not progressed as that of ICSS (Liebman 1985).

### ICSS in Various Species

Many investigators have reported the presence of self-stimulation behavior in various species and this has strengthened the observations and confirmation made by Olds and Milner (1954). It has been shown that ICSS is not an idiosyncrasy of rat, but can be demonstrated in many other animals including rabbits (Bruner 1966), cats (Wilkinson & Peele 1963), dogs (Stark & Boyd 1963), pigs (Baldwin & Parrott 1978), goats (Persson 1962), dolphins (Lilly & Miller 1962), rock squirrels (Wetzel & King 1966), monkeys (Porter et al. 1959, Routtenberg et al. 1971), chicks (Andrews 1967), snails (Balaban & Chase 1989) and humans (Porter et al. 1959, Heath 1963, Stellar & Stellar 1985, Bishop et al. 1963, Sem-Jacobsen & Styri 1972, Delgado et al. 1954).

### ICSS and Natural Rewards

A major reason for the initial enthusiasm of behavioral neuroscientists about the ICSS experiments was that they seemed to provide an approach that made it possible to deal directly with the neural mechanisms of reinforcement; and it was assumed that the ICSS and natural rewards like food, water and sex were essentially similar (Olds 1958). However, there were a number of observations that indicated possible differences. Feeding is a self-sustaining and sex is a life-preserving behavior on which the existence of the individual and the species depends respectively. However, it is surprising that reinforcing effect of ICSS is so potent that a starving rat will often choose electrical stimulation for which it has been trained (Rao 1985). Routtenberg (1978) trained rats to press one lever for brain electrical stimulation, while the other for food pellets, thus giving the choice between the electrical stimulation and the food. The animals selected the lever that administered electrical stimulation rather than the other lever, which dispensed food pellets.

### Neural Substrates of ICSS

Hypothalamus and limbic system hypothesis of ICSS for central integration of motivation was proposed by Olds (1962). Original mapping studies suggested that the MFB was the anatomical substrate of ICSS. This was followed by catecholaminergic hypothesis (German & Bowden 1974). In the later years, efforts were made to delineate the role of noradrenergic and dopaminergic hypotheses. Stein's noradrenergic hypothesis was subsequently elaborated as anhedonia hypothesis based on noradrenaline (NA) mechanisms of reward systems (Stein & Wise 1969, Stein 1975). It was then superceded by the dopamine (DA) hypothesis of anhedonia (Wise & Rompre, 1989). Routtenberg (1978) described both the NA and DA pathways of reward system in the rat brain. Stein and Beluzzi (1979) proposed that endorphins might be important for the reward system, as opioid peptides and their receptors are densely distributed in the hypothalamus, nucleus accumbens, VTA and locus coeruleus, and the regions from where ICSS is obtained.

Neuropsychopharmacological studies have implicated dopaminergic or noradrenergic or both pathways that ascend within the MFB to constitute the basis of neural substrate of positive reinforcement of ICSS. Further work indicated that both ascending and descending non-catecholaminergic fibers and neurons of opioid peptides as well as non-opioid peptides like cholecystokinin (CCK) and neurotensin (NT) are also involved in the ICSS reward mechanisms (Stein 1985, Singh 1990, Singh et al. 1994). The evidence pointing to DA as the critical neurotransmitter in neuronal systems supporting ICSS has come from the studies of Phillips and Fibiger (1978), Clavier and Gerfen (1979), Phillips et al. (1982), Wise (1980b) and Carey (1986). Anatomically the sites supporting ICSS are closely related to mesocortico-limbic DA system. This system has its cell bodies in the VTA and the axons run along the MFB to the nucleus accumbens (nACB) and also to medial frontal cortex. Most, if not all sites in the meso-cortico-limbic system have been shown to support ICSS. Evidences from pharmacological studies also support the critical function of DA in ICSS; DA receptor antagonists decrease ICSS responses from LH-MFB as well as SN-VTA in a dose-related manner.

The involvement of intrinsic neurons of LH has been shown in ICSS (Velley et al. 1983, Nassif et al. 1985, Lestang et al. 1985, Velley 1986, Velley et al. 1988). It has added another component to the neural substrate of ICSS. The role of intrinsic neurons

has been further demonstrated by Takeichi et al. (1985) in the ICSS of ventral tegmentum and by Mora and Ferrer (1986) in the ICSS of medial prefrontal cortex. The emerging picture then, is of a reward circuit involving neurons of descending myelinated fiber system in the MFB, communicating with dopaminergic neurons of the VTA and ultimately resulting in the synaptic release of dopamine at the terminals in nucleus accumbens where the reinforcing effects of psychomotor stimulants are pharmacologically initiated.

## Neurochemical Basis of ICSS

### *Noradrenergic System and ICSS*

Earlier theories have suggested that NA was a primary neurotransmitter involved in reinforcement processes (Stein 1964). This belief stemmed from the observation that there is a loose correlation between ascending noradrenergic projection and brain areas that support ICSS.

### *Anatomical Localization Studies*

The main anatomical support for the NA hypothesis was derived from mapping studies wherein the ICSS was shown in the locus coeruleus (LC) and its primary ascending outflow i.e., the dorsal noradrenergic bundle (DNAB) to the telencephalon (Amaral & Routtenberg 1975) and in some sites like LH and medial prefrontal cortex that contain NA synapses (Stein 1975). It is also observed that not all the regions that receive projections from the noradrenergic system support the ICSS. While the cingulate, entorhinal and pyriform cortices and hippocampus are known to support ICSS (Routtenberg et al. 1971) but other regions receiving LC innervation do not. Thus, the structures innervated by the NA system alone may not be the possible substrate for ICSS. However, the concentration of NA was increased in the hippocampus and motor cortex following ICSS of LH-MFB and SN-VTA (table 3) (Shankaranarayana Rao 1993, 1996a, 1998b, 1998d).

### *Pharmacological Studies*

Wise and Stein had earlier provided evidence for NA hypothesis demonstrating decreased ICSS with disulfiram and diethyl-dithio-carbonate (inhibitors of dopamine β-hydroxylase, DβH). This effect could be reversed by subsequent intraventricular injection of NA. However, the later findings of Rolls et al. (1974) pointed out that disulfiram had no primary effect upon ICSS and it would disrupt ICSS presumably because of the induction of sedation and sleep or reduction of arousal as measured by locomotor activity and rearing. Further, FLA-63 (a non-sedating DβH inhibitor) and U-14624, (a sedative type DβH inhibitor) when used at a dose that had been shown to reduce the central NA level by 70% and to cause nearly complete inhibition of NA synthesis, did not cause any significant effect on LH-ICSS. This was considered as most important pharmacological evidence against NA hypothesis. Based on these findings, the catecholominergic hypothesis of reward was modified by including dopamine (Crow 1972).

**Table 3** *ICSS induced alterations in the levels of neurotransmitters (ng/g tissue) and AChE activity (X10$^{-6}$ μmoles hydrolyzed/min/g tissue) in the hippocampus and motorcortex*

| Neuro-transmitters | Group | Hippocampus | Motor cortex |
|---|---|---|---|
| Noradrenaline | Control | 242.85 ± 24.12 | 300.15 ± 17.60 |
| | ICSS | 325.83 ± 36.75* | 376.18 ± 13.26* |
| Dopamine | Control | 178.28 ± 19.65 | 291.33 ± 9.77 |
| | ICSS | 270.10 ± 22.59* | 382.85 ± 13.07* |
| 5-Hydroxy-tryptamine | Control | 364.60 ± 27.02 | 420.99 ± 13.99 |
| | ICSS | 332.84 ± 27.40 | 437.53 ± 25.99 |
| Glutamate | Control | 9.44 ± 2.01 | 8.82 ± 1.54 |
| | ICSS | 16.77 ± 2.66* | 14.09 ± 1.80* |
| GABA | Control | 2.37 ± 0.66 | 2.42 ± 1.21 |
| | ICSS | 3.54 ± 0.91 | 3.61 ± 0.48 |
| AChE activity | Control | 6.15 ± 1.17 | 6.16 ± 1.52 |
| | ICSS | 17.24 ± 1.99* | 11.97 ± 1.83* |

**Each value represents mean (± SD) data of 6 rats per group. *p<0.001 (one-way ANOVA) (Shankaranarayana Rao 1996).**

### *Dopaminergic System and ICSS*

Evidence for the involvement of dopaminergic system in ICSS has been obtained from six major observations.

### *Mapping Studies*

A close correlation exists between the highly responding loci of ICSS in the SN-VTA and the location of DA neurons in this region. The dorsal, ventral and lateral boundaries of positive regions for ICSS correspond precisely to the boundaries of SN and VTA dopaminergic neurons. The lower ICSS

threshold was found in the areas of ventral midbrain, where the dopaminergic fibers gather and begin to ascend up (Corbett & Wise 1979, 1980, Wise, 1981). The probability of eliciting ICSS from subdivisions of mesolimbic system ranges from 78-100%, compared with a probability of 8% for sites outside catecholominergic systems. The highest probability of 95-100% is obtained from the terminal structures where the system has least overlap with other catecholaminergic systems; hence it would appear that the mesolimbic DA system is responsible for the major component of ICSS.

### *Drug Self-Administration Studies*

The second major line of evidence suggesting the role of dopaminergic system in brain stimulation reward is from studies of self-administration of drugs. During ICSS, rats vigorously self-administer DA or its agonists through oral, intravenous or intraventricular or intracerebral route (Baxter et al. 1974, Wise 1980a) into the nucleus accumbens (Hoebel et al. 1982), or into medial prefrontal cortex (Goeders & Smith 1983). This can be blocked by systemic injection of neuroleptics (Bozarth & Wise 1982, Ettenberg et al. 1982) or by lesions of dopaminergic terminal in the nucleus accumbens. Humans self-administer DA agonists like amphetamine and cocaine (DA uptake inhibitor), and report the subjective feelings of pleasure. In humans, the reported euphoric properties of amphetamine are reduced when the subjects are given neuroleptic pretreatments.

### *Lesioning Studies*

ICSS of VTA was greatly attenuated by 6-hydroxydopamine (6-OHDA)-induced lesions of ascending meso-telencephalic DA pathways (Phillips & Fibiger 1978, Fibiger et al. 1987). ICSS of VTA was reduced after lesioning of nigrostriatal projection by 6-OHDA, which attributed to motor deficits (Clavier & Fibiger 1977). 6-OHDA lesion of ventral striatum, a site that primarily carries the terminals of mesolimbic DA projections, reduced the LH-ICSS, while introduction of crystalline DA in the damaged site restored the LH-ICSS (Neil et al. 1982). Similarly, blocking of DA receptors in nACB by spiroperidol not only reduced the VTA-ICSS, but also antagonized the intravenous self-administration of amphetamine (Phillips et al. 1983). The role of SN-VTA on ICSS was further strengthened by the findings that destruction of dorsal striatum unilaterally by kainic acid significantly increased the current intensity thresholds for SN-ICSS, but not in VTA-ICSS (Phillips et al. 1982). Clavier and Gerfen (1979) showed that destruction of VTA with 6-OHDA abolished the ICSS of ipsilateral medial prefrontal cortex, which has terminals of the DA neurons of VTA. Phillips and Fibiger (1978) observed that the ipsilateral 6-OHDA lesions of SN disrupted both the ipsilateral and contralateral LH-ICSS equally. Koob et al. (1977) did not show such contralateral effect. Electrolytic lesions of VTA reduced ipsilateral LH-ICSS (Boyd & Gardner 1967).

### *Biochemical Studies*

Increased turnover of DA has been observed in both nACB and caudate putamen (CPU) of mice after the stimulation of DA fibers at the level of the hypothalamus (Garrigues & Cazala 1983) and VTA (Van Heuven-Nolson et al. 1983). A similar increase in the metabolism of DA was observed in the nACB, CPU and olfactory tubercle (OT) of rats following ICSS of the VTA (Fibiger et al. 1987). Using in vivo microdialysis methodology, Nakahara et al. (1989) have shown an increase in the DA and 5-HT metabolism in nACB produced by ICSS of MFB. However, ICSS of hypothalamus, which also gives equally high rates of ICSS, did not produce this type of increase in DA metabolism in nACB, CPU and OT (Mitchell et al. 1982). Our recent studies have shown an increase in the concentration of DA in hippocampus and motor cortex following 10 days of ICSS from LH-MFB and SN-VTA (table 3) (Shankaranarayana Rao et al. 1998b, 1998d).

### *Pharmacological Studies*

*Dopamine agonists:* The effects of cocaine and amphetamine on ICSS have been extensively studied. Both are indirect dopamine agonists that increase synaptic dopamine by different mechanisms. Cocaine inhibits the reuptake of dopamine into presynaptic terminals (Heikkila et al. 1975, Izenwasser et al. 1990), while amphetamine stimulates dopamine release (Uretsky & Snodgrass 1977, Schwartz et al. 1980), enhances the response rate (Stein 1964, Crow 1969, Greenshaw et al. 1983) and lowers the stimulation threshold (Zarevics & Setler 1979, Cassens & Mills 1973, Wauquier & Niemegeers 1974, Schaefer & Holtzmen 1979, Esposito et al. 1980, Hubner

et al. 1987). Phillips et al. (1989) have attempted to measure the neurochemical correlations of ICSS directly and found that DA levels in the MFB are increased following the stimulation of VTA. Additionally, cocaine and GBR 12909, highly selective dopamine uptake inhibitors, facilitate self-stimulation and concomitantly increase extracellular DA.

*Dopamine antagonists:* There have been many reports showing that the DA antagonists either attenuate or abolish the reinforcing effects of brain stimulation. Neuroleptic drugs such as pimozide, haloperidol and cis-flupenthinol attenuate ICSS by lowering the response rate or increasing the threshold (Zarevics & Setler 1979, Fouriezos et al. 1978, Fouriezos & Wise 1976, Eteenberg & White 1981, Gallistel et al. 1982, Stellar et al. 1983). These drugs indeed reduce the reinforcement value such as food, water and brain stimulation (Ettenberg, 1989).

The efficacy of neuroleptic drugs in suppressing brain stimulation appears to be correlated with their affinity for D2 but not D1 dopamine receptors (Gallistel & Davis, 1983). There are however, reports suggesting that D1 receptors be also involved in reinforcement. Systemic administration of SCH-23390, a selective D1 receptor antagonist, reduces response rate for ICSS (Nakajima & McKenzie, 1986). Administration of SCH-23390 into the nucleus accumbens produces a decrease in the response of VTA for stimulation (Kurumiya & Nakajima, 1988), while sulpiride, a D2 antagonist, has no effect. Singh et al. (1996, 1997b) have reported that haloperidol induced inhibition and apomorphine induced facilitation of ICSS of SN-VTA, when these were injected into LH-MFB. In contrast, the injection of either haloperidol or apomorphine into SN-VTA supressed its ICSS, while caused no effect on the ICSS of LH-MFB.

### *Neuronal Transplantation Studies to Reconstitute ICSS Behavior*

Fray et al. (1983) have destroyed the DA neurons in SN-VTA by 6-OHDA and then transplanted fetal SN tissue into forebrain above the caudate putamen. Then the electrodes were implanted in the grafted region and ICSS was demonstrated in these animals. They also exhibited impressive rate-intensity function. Rao (1996) has demonstrated the recovery of ICSS response (which was abolished by prior lesioning) following transplantation of embryonic ventral mesencephalic tissue in lesioned adult SN-VTA area.

In summary, the dopaminergic system appears to be a major neurochemical substrate in ICSS of various brain regions.

### *Serotonergic System and ICSS*

Traditionally serotonin (5-Hydroxytryptamine, 5-HT) has not been thought to be the neurotransmitter involved in the mediation of ICSS behavior. Theories of reinforcement have emphasized the action of an inhibitory or aversive 5-HT system. Studies employing LH-ICSS generally show an increase in the ICSS after para-chlorophenylalanine (PCPA) treatment (Poschel & Ninteman 1964, 1966, Van Der kooy et al. 1977). Similarly, the neurotoxic lesion of 5-HT neurons by intraventricular injection of 5-6-dihydroxytryptamine increases LH-ICSS and the administration of 5-HT precursor, 5-hydroxy-tryptophan depresses LH-ICSS (Poschel et al. 1974). ICSS in forebrain areas innervated by 5-HT neurons, such as striatum and hippocampus is suppressed after PCPA treatment (Van Der kooy et al. 1977).

Nakahara et al. (1989b) using in vivo microdialysis, showed an increase in the level of serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in nucleus accumbens during MFB-ICSS. This suggests that SS of the MFB in rats cause an increase in serotonin release in the nACB and such changes may be involved in mediating SS of the MFB. Further studies are required to evaluate the involvement of serotonergic system in brain stimulation reward. Our recent studies have demonstrated that 10 days of ICSS experience from LH-MFB and SN-VTA does not cause any significant alterations in the level of 5-HT in the hippocampus and motor cortex (table 3) (Shankaranarayana Rao et al. 1998b,d).

### *Glutamatergic System and ICSS*

Recent work on excitatory aminoacid (EAA) neurotransmitters offers a new lead with regards to the role of this transmitter system in ICSS. Receptors sensitive to N-Methyl D-aspartate (NMDA) have been implicated in a variety of important behavioral functions, including learning and memory (Collingridge 1987, Danysz et al. 1988, Morris et al. 1986), imprinting (McCabe & Horn 1988) and spontaneous locomotor activity (Boldry & Uretsky, 1986). The involvement of EAA in learnt behavior raises

the possibility of a primary role in self-stimulation (Herberg & Rose, 1989, 1990). EAA projections have infact been traced from the rat frontal cortex to major reward sites like the nucleus accumbens and ventral tegmentum (Christie et al. 1985). Glutamate/aspartate is appropriate as a transmitter to perform this role since NMDA as well as non-NMDA receptors could mediate both the functions assigned to self-stimulation pathway (Gallistel 1973). It may result in the establishment of a long-term record of ongoing activity as exemplified by effects of NMDA in cortex and hippocampus (Artola & Singer 1987, Collingridge 1987).

Preliminary tests, however have failed to produce any impairment of SS after moderate doses of indirect NMDA antagonists, including MK-801 and ketamine (Anis et al. 1983, Wong et al. 1988, Herberg & Rose 1989). Nevertheless, some degree of suppression was obtained with intraperitonial injections of kynurenic acid (Herberg & Rose 1989), a non-selective antagonist of kainate and quisqualate as well as NMDA receptors (Ganong et al. 1983). The reduction of SS produced by kynurenic acid, despite its rather poor penetration of the blood-brain barrier, provides tentative evidence for the possible role of EAA receptors in the reinforcement process. Recent studies by Shankaranarayana Rao et al. (1995b, 1998b, d) have showed an increase in the level of glutamate in the hippocampus and motor cortex following 10 days of ICSS experience (table 3). Pretreatment with ketamine, a non-competitive antagonist of the NMDA receptor blocked the facilitatory effect of medial prefrontal cortex stimulation on subsequent ICSS acquisition. This finding provides further support for the view that the NMDA receptor is involved in mechanisms of behavioral plasticity.

### *GABAegic System and ICSS*

Zarevics and Setler (1981) assessed the effects of systemic administration of picrotoxin (blocker of $Cl^-$ ionophore of GABA complex) and muscimol (agonist of GABA-A) on MFB-ICSS. Picrotoxin produced an elevated ICSS threshold and thus a decreased reward. In contrast, muscimol lowered the ICSS threshold and thus increased the reward. Nazzaro and Gardner (1980) tested the effects of nalaxone and picrotoxin on rate-dependent ICSS threshold titration paradigm for VTA-ICSS. Nalaxone did not affect ICSS threshold but the picrotoxin caused a significant lowering of SS threshold. Fenton and Liebman (1982) reported the decrease of LH-ICSS with systemic administration of baclofen, a putative GABA- B agonist. The GABA antagonist's bicuculline and picrotoxin were found to decrease the response rate for self-stimulation (Kent & Fedinets, 1976). When a rate-independent threshold procedure was used, picrotoxin lowered the threshold for VTA stimulation, an effect that was not blocked by nalaxone (Nazzaro & Gardner, 1980). Porrino and Coons (1980) however reported the opposite effect, that picrotoxin increases the ICSS threshold. The animals in this study apparently exhibited marked stereotypical behaviours that may have interfered with their ability to respond to the stimulation. Using a two-lever reset method to determine ICSS threshold. Zarevics and Setler (1981) have found an increase in threshold with picrotoxin and a decrease with GABA-mimetic musimol. Since there is a tonic inhibition by GABA on dopamine neurons, blockade of this inhibition by a GABA antagonist results in increased dopaminergic activity.

The effects of anxiolytic drugs on ICSS have been extensively studied. Meprobamate (Olds & Travis 1960), Chlordiazepoxide (Lorens & Sainati 1978, Domino & Olds 1972, Panskepp et al. 1970, Ichimaru et al. 1983), diazepam (Ichimaru et al. 1983), pentobarbital (Seeger et al. 1981, Olds & Travis, 1960, Herberg & Williams 1983, Mogenson 1964) and clonozepam (Herberg & Williams 1983) all increased the ICSS behavior when appropriate conditions and doses were used. Rompre and Wise (1988) reported an increase in the ICSS threshold with musimol into VTA and finally the inhibition of MFB-ICSS. Singh et al. (1997a) have reported that an intra LH-MFB injection of bicuculine supress its ICSS and also ICSS of SN-VTA, where as intra SN-VTA injection cause no change in its ICSS. On the contrary, injection of picrotoxin into SN-VTA inhibited ICSS of SN-VTA and LH-MFB.

### *Cholinergic System and ICSS*

Cholinergic cells of the pedunculopontine tegmental nucleus (Ch5) and lateral tegmental nucleus (Ch6) send axons to the substantia nigra (A9) and ventral tegmental area (A10) (Beninato & Spencer 1987, Clarke et al. 1987, Fujimato et al. 1990, Niijima & Yoshida, 1988, Tokumo et al. 1988, Woolf et al. 1990). This is the only known cholinergic input to these

dopamine cells (Woolf, 1991). These cholinergic terminals make many asymmetric contacts onto the dendrites and cell bodies of DA cells. Newman (1972) tested a range of cholinergic agonists and antagonists like atropine sulfate, pilocarpine hydrochloride, scopolamine (centrally and peripherally active ACh-M antagonist), atropine methylnitrate (peripherally active ACh-M antagonist), and nicotine tartarate (Centrally and peripherally active ACh-N agonist). He observed that nicotine caused an enhancement of ICSS from both LH and SN-VTA. On the contrary, atropine was found to cause facilitation of LH-ICSS at lower doses and inhibition at higher doses. Wauquier and Rolls (1976) reported the enhancement of LH-ICSS with atropine sulfate. Similarly, Pradhan and Kamath (1973) reported facilitation of LH-ICSS with atropine. In contrast, Rao (1985) and Singh (1990, 1997a) demonstrated a reduction of LH and SN-VTA response with atropine sulfate. Gratton and Wise (1985) showed that the atropine blocked the contribution of the fast fiber system MFB-ICSS in a psychophysical study.

Redgrave and Horrel (1976) showed 50% facilitation in the ICSS of LH-MFB, when ACh was injected into the SN-VTA. Singh (1990) reported an increase in self-stimulation responses in LH-MFB and SN-VTA sites when carbachol (ACh-M receptor agonist) was injected into these sites. Yeomans et al. (1985) studied the effects of intra-VTA injections of carbachol and atropine on LH-ICSS, using the conditioned place preference paradigm. The rats preferred the compartments in which they received carbachol. They concluded that ACh-M receptors in VTA seemed to be critical for the ICSS of LH-MFB. Our recent studies have shown that the activity of AChE increased after ICSS rewarding experience (table 3) (Shankaranarayana Rao et al. 1995b, 1998b, 1998d). There have been other studies aimed at defining the role of ACh in mediating ICSS. Domino and Olds (1968) showed that physostigmine leads to an increase in brain ACh levels and a decrease in ICSS response rate. Conversely, atropine and scopolamine, both selective muscarinic antagonists, raise the threshold for ICSS (Kornetsky & Bain, 1990, Kofman et al. 1990). Like nicotine, ACh is known to interact directly with dopaminergic neurons to stimulate DA release (Giorguieff et al. 1976). Thus, it is possible that ACh may modulate reinforcement indirectly via the modulation of dopaminergic activity (Yeomans et al. 1993).

### *Opiate System and ICSS*

Opiates have long history of use and abuse in diverse cultures as painkillers and euphoric agents leading to addiction (Engel & Oreland 1987, Smith & Lane 1983). The positive reinforcing properties of morphine and opiates were first reported by Spagg (1940) who showed that chimpanzees previously made physically dependent on morphine could learn to select one of the two boxes that had been baited with syringe filled with morphine solution for administration. Although it has long been known that humans and non-human species will administer opiates via systemic route, only in recent years its mode and site of action in the CNS being explored and understood (Esposito & Konetsky 1977, Smith & Dworkin 1986, Stein & Belluzzi 1987, Wise 1987, Koob & Bloom 1988). Rats and monkeys known to self-administer opiates and it were also observed that single dose of opiates could lower the threshold for ICSS (Belluzzi & Stein 1977, Mello & Mendelzon 1979, Stein & Belluzzi 1979). Amalric et al. (1987) have showed positive reinforcement properties of β-endorphin in a conditioned place preference paradigm and suggested that its reinforcing effect is most probably mediated by μ and δ opioid receptors.

Direct injections of morphine and other opiates, and opioid peptides reinforcing when administered into the regions of the dopaminergic neurons of VTA (Van Ree & deWied 1980). This was also observed in a conditioned place preference paradigm wherein animals learn to return to the place where they had experienced with opiates in the past (Phillips & LePiane 1980, 1982, Bozarth & Wise 1982, 1984) and also in a lever pressing self-administration paradigm where responding is reinforced by direct intra-VTA injections of morphine (Bozarth & Wise 1981) or fentanyl (Van Ree & deWied 1980) or met-enkephalinamide (Broekkamp et al. 1979). Lower doses are effective in VTA than are required for other regions including nucleus accumbens. Microinjections of opiate antagonists into VTA cause compensatory increases at low doses, but extinguish the responding for intravenous opiates at high doses (Britt & Wise 1983). Singh et al. (1994) have showed facilitation of ICSS of SN-VTA by

microinjections of opioid receptor agonists. Microinjections of thiorphan (enkephalinase inhibitor) produced a reinforcing state in the place preference paradigm, presumably mediated by endogenous opioid peptide transmitter (Glimcher et al. 1984). The reinforcing action of opiates seems to be localized to the regions of the DA containing neurons that are implicated in psychomotor stimulant reward (Bozarth 1986, 1987, Wise 1987).

Thus, various neurotransmitter systems are found to be involved to varying degrees in the regulation of ICSS. Eventhough, the DA system appears to be a major component in mediating ICSS behavior, it is very difficult to assign an exclusive role for a single neurotransmitter in the production of ICSS. Further studies are required to evaluate the interaction of excitatory and inhibitory neurotransmitters, which may determine the final expression of ICSS.

## ICSS, a Model to Study Neuronal Plasticity

### *ICSS Induced Neuronal Plasticity*

Neuronal plasticity is an important property of the brain and refers to morphological, biochemical and physiological changes occurring in both the adult and developing nervous system. Although neuronal circuitry is capable of undergoing different types of changes, synaptic changes are the most critical ones because they alter communication between neurons. Synaptic plasticity pertains to changes in the complex synaptic networks of individual brain circuits in response to various interventions. The capacity of the nervous system to reorganize the pattern of synaptic connectivity needs to be understood, because it can potentially bring about changes in the behavior following neural injury.

Recent progress in our understanding of neuronal plasticity also confirms that patterns of neuronal connectivity are not rigid. Merzenich and Kaas (1982) demonstrated that the sensory maps can be redrawn in adult brains and suggested that the brain is a network that is continually remodelling itself. Their findings brought everybody's attention to the idea that plasticity occurs in the adult nervous system and inferred that the adult brain can reorganize itself in areas that were long thought to be completely hard-wired. Cortical motor and sensory neurons are not permanently fixed in the way they subserve their limb functions. On the contrary, they quickly adapt to changing demands. In the adult and developing animals and in humans the topographic maps of sensory and motor neuronal representations are capable of physiological and structural reorganisation.

Recently, the electrical self-stimulation paradigm has proved to be very useful in delineating the neural substrate involved in learning and drug abuse. Self-stimulation involves operant learning, which can induce changes in the neuronal cytoarchitecture. Accordingly, we have used this experimental paradigm to study learning related neuronal plasticity. We have conducted a series of experiments to determine the self-stimulation (SS) rewarding experience induced neuronal plasticity in hippocampal and motor cortical neurons.

### *ICSS Induced Dendritic Plasticity*

Dendritic outgrowth in hippocampal and motor cortical neurons in adult mammals is influenced by various factors such as nutritional status, hormones, aging, ethanol-consumption, brain lesions and injury, social isolation, sensory deprivation, exposure to complex or enriched environment, training for various tasks and electrical stimulation (Shankaranarayana Rao 1996, 1997, 1998). The electrical self-stimulation has been considered as one of the intensely rewarding behavioral experience, perhaps even more influential than feeding or sexual rewards (Olds 1962). Self-stimulation rewarding experience for 10 days resulted in an increase in the dendritic branching and dendritic length in CA3 hippocampal and layer V motor cortical pyramidal neurons (figure 2) (Shankaranarayana Rao et al. 1992, 1993, 1994). The CA3 pyramidal neurons were selected because they form an essential link in the hippocampal trisynaptic circuit, implicated, as a substrate for learning and memory and CA3 field appears to be the major focus of integration of intrahippocampal activity. The layer V motor cortical neurons were studied because they include the projection neurons, whose axons terminate on motor neurons in the spinal cord and which control the movement of forelimbs and thought to be an integrator, by providing a final common pathway for the self-stimulation related motor activity i.e., pedal pressing. The structural changes observed following ICSS seems to be specific to hippocampal and motor cortical pyrami-

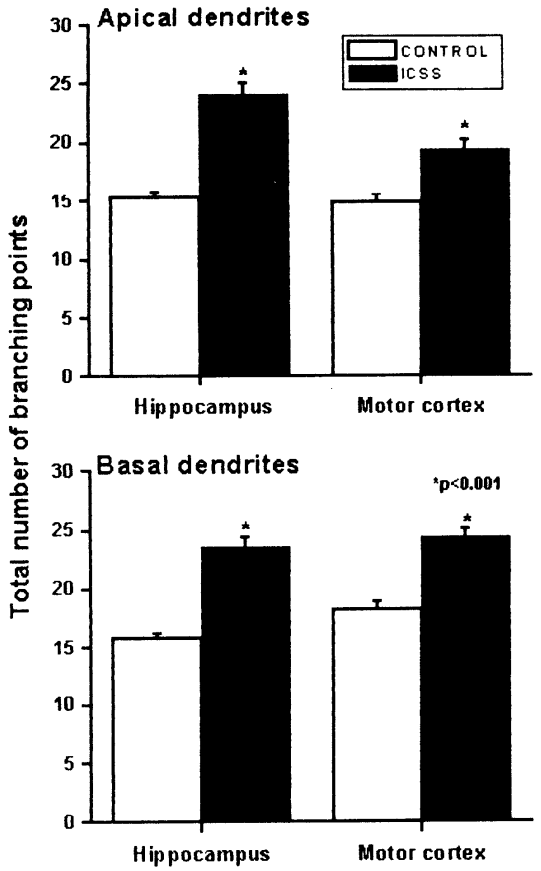


Figure 2 Number (mean ± SD) of branching points in apical and basal dendrites in CA3 hippocampal and layer V motor cortical pyramidal neurons from control and ICSS experienced groups of rats. Note a significant (*p<0.001, two-way ANOVA) increase in the number of branching points in ICSS rats compared to control (Shankaranarayana Rao 1996c).

dal neurons. Because our preliminary studies have showed that ICSS does not produce structural changes in amygdala neurons (Shankaranarayana Rao et al. unpublished observations). It indicates that ICSS induced structural changes are specific to certain areas of the brain and it is not a general phenomenon. Thus the ICSS provides an excellent model to evaluate the cellular basis of the learning and memory.

In addition to the dendritic growth, the size of brain regions is known to be altered significantly by different experiences. For example, Walsh et al. (1972) observed the medial area of the hippocampus was significantly thicker in animals reared in complex environments than those reared in isolation. Rosenzweig (1962) also reported that there was an increase in the size of hippocampus as a consequence of exposure to complex environment. Our studies have also revealed a significant increase in the thickness of lacunosum, radiatum and lucidum laminae in the CA3 region of the hippocampus in self-stimulation experienced rats (Shankaranarayana Rao et al. 1993).

Dendritic outgrowth in adults can also occur by electrical stimulation. Rutledge et al. (1974) have observed an additional outgrowth of apical dendrites in the cortex of adult cats after long-term electrical stimulation applied to the left suprasylvian gyrus. After this period, it was observed that apical dendrites of layer II and III pyramidal neurons were shown to have significantly more dendritic branches and spines in the cortex contralateral to the stimulated side. They interpreted this as evidence that increased use of specific pathways to and within the cerebral cortex of adult cats can induce postsynaptic growth.

Greenough et al. (1985) have reported that training the rats to reach for food, results in increased branching of apical dendrites of layer V pyramidal neurons in the anterior cortical region, which are involved in sensory and motor innervation of the contralateral forelimb of the rat. Similar changes have also been observed in layers II-III pyramidal cell basilar dendrites in rat motor-somatosensory forelimb cortex (Withers & Greenough 1989). Jones and Schallert (1994) have demonstrated an use-dependent dendritic growth in layer V pyramidal neurons of the motor cortex. In addition to the effect of various environmental factors, the treatment of (-) deprenyl, a selective monoamine oxidase-B inhibitor resulted in a significant increase in the dendritic arborization of CA3 hippocampal and layer III prefrontal cortical neurons in monkey brain (Lakshmana et al. 1998, Shankaranarayana Rao et al. 1999).

### *ICSS Induced Alterations in the Density of Dendritic Spines*

Dendritic spines are the major postsynaptic targets for excitatory synaptic inputs onto principal neurons in the hippocampus, the neocortex and other brain regions. Dendritic spines have been proposed to constitute the main locus of long-term synaptic modification associated with functional plasticity in neurons of the central nervous system. The density of dendritic spines over any given length is subjected to wide fluctuation depending upon variety of phenomena in hippocampal and motor cortical neurons. Extensive analyses of hippocampal neurons has indicated that the dendritic spines undergo marked morphological changes following exposure

to various stimuli, including tetanic stimulation, kindling or even natural or hormonal stimulation.

Our previous studies have demonstrated the self-stimulation of lateral hypothalamus (LH) and substantianigra-ventral tegmental area (SN-VTA) for 10 days results in significant increase in the number of dendritic branching and dendritic intersections in CA3 hippocampal and layer V motor cortical pyramidal neurons (Shankaranarayana Rao et al. 1993, 1994). Accordingly, the present study was carried out to evaluate the alterations in dendritic spine density in the different categories of the dendrites of CA3 hippocampal and layer V motor cortical pyramidal neurons induced by self-stimulation experience.

ICSS rewarding experience resulted in a significant increase in numerical density of dendritic spines in different categories of both apical and basal dendrites in CA3 hippocampal and layer V motor cortical pyramidal neurons (table 4) (Shankaranarayana Rao et al. 1994, 1995a, 1999a) and also an increase in the numerical density of thorny excrescences in apical dendrites of CA3 neurons of the hippocampus (Shankaranarayana Rao et al. 1998a). We have earlier reported that self-stimulation behavior elicited from LH and SN-VTA for a period of 10 days resulted in an increase in the dendritic branching and intersections of the CA3 hippocampal and layer V motor cortical pyramidal neurons (Shankaranarayana Rao et al. 1993, 1994). Increased dendritic arborization is coupled with an increase in the spine density observed in the present study can considerably enhance the postsynaptic receptive area of these neurons.

**Table 4** *Mean number of dendritic spines per 76μm in different categories of apical and basal dendrites in CA3 hippocampal (a) and layer V (b) neurons of the motor cortex*

| | Dendritic category | Control | ICSS |
|---|---|---|---|
| ***(a) CA3 pyramidal neurons of the hippocampus*** | | | |
| Apical dendrites | Main shaft | 51.35 ± 2.98 | 63.01 ± 2.66** |
| | Sub branch | 47.06 ± 3.45 | 58.88 ± 3.10* |
| | Oblique shaft | 52.70 ± 4.93 | 74.10 ± 4.17** |
| | Primary branch | 53.30 ± 3.47 | 84.03 ± 3.91** |
| Basal dendrites | Main shaft | 46.36 ± 2.30 | 63.68 ± 1.84** |
| | Primary branch | 58.50 ± 3.93 | 80.80 ± 3.05** |
| ***(b) Layer V pyramidal neurons of the motor cortex*** | | | |
| Apical dendrites | Main shaft | 40.26 ± 2.28 | 76.56 ± 3.17** |
| | Oblique shaft-I | 52.23 ± 4.37 | 73.93 ± 4.66** |
| | Oblique shaft-II | 53.80 ± 5.59 | 80.76 ± 5.08** |
| | Primary branch | 56.48 ± 5.87 | 80.66 ± 5.21** |
| Basal dendrites | Main shaft | 45.91 ± 3.57 | 62.46 ± 3.04** |
| | Primary branch | 51.70 ± 5.58 | 69.81 ± 3.84** |

Each value represents mean (± SD) data of 6 rats per group. *p<0.005; **p<0.001 (two-way ANOVA) (Shankaranarayana Rao 1996).

### *Spine Density Changes in the Hippocampus*

The apical dendrites of CA3 pyramidal neurons receive dentate neuronal mossy fibers at the proximal segments (stratum lucidum), entorhinal fibers at stratum moleculare, septo-hippocampal fibers at the middle and terminal dendritic regions (stratum radiatum) and other associational, commissural and collateral inputs in the middle segments. The present study showed an increase in the spine density at the proximal dendritic segments, where the mossy fibers terminate and middle segments, where the medial septal, commissural, collateral and short axon endings synapse, as also probably the entorhinal terminals on to the primary branches. While basal dendrites of CA3 neurons are found to receive mossy fiber collaterals, commissural and septal inputs at the proximal dendritic regions (stratum orience). Our results showed a significant ($p<0.001$) increase in dendritic spine densities in these regions of basal dendrites. Hence, self-stimulation might be enhancing the synaptic transmission in these specific pathways to the particular layers of the CA3 neurons, resulting in an increase in the density of spines.

Dendritic plasticity in hippocampal neurons has been reported with development, learning experience and aging. Recent studies have shown training-induced changes in dendritic spine density in different regions of the brain and also stimulation of specific brain pathways causes various structural modifications in post synaptic specializations, dendritic spines as well as synapses in hippocampal neurons. Ben-Ari and Repressa (1992) reported brief high frequency stimulation induce long-term potentiation (LTP) and mossy fiber sprouting in the hippocampus and they suggested the usage-dependent structural rearrangement of the neuronal network, a model for plasticity in the adult nervous system. An increase in the population spikes in the dentate gyrus induced by perforant path stimulation has been observed on completion of learning. Mitsuno et al.(1994) have reported the training induced poten-

tiation of population spikes in CA3 neurons occurred with the advance of learning and suggested that LTP in CA3 neurons induced by learning may be related to the memory storage. The LTP can also set in motion, a cascade of events that includes changes in gene expression, sprouting of fibers and the establishment of new synaptic contacts.

### *Spine Density Changes in the Motor Cortex*

The SS experience results an increase in dendritic spine density in layer V pyramidal neurons of the motor cortex. This increase may be partly related to the vigorous motor activity (pedal pressing) exhibited during SS sessions. This probably indicates an increase in afferent inputs to these motor cortical neurons from the sensory-motor pathway as well as other circuits involved in planning and execution of motor activity. Rutledge et al. (1974) have reported an increase in dendritic branching and spine densities after a long-term repetitive electrical stimulation of the presynaptic pathways of layers II and III pyramidal neurons in the adult cat cortex and suggested that increased use of pathways to the cerebral cortex will produce post synaptic growth in cortical neurons. Our previous studies have demonstrated that the self-stimulation of LH and SN-VTA for 10 days results an increase in dendritic branching and intersections in layer V pyramidal neurons of motor cortex (Shankaranarayana Rao et al. 1993, 1994), which was accompanied by an increase in the dendritic spines.

Dendritic plasticity of cortical neurons has been reported to be influenced by the nutritional status, environmental complexity and differential experiences and training. Our results indicates a post synaptic modifications and synaptic reorganization after pedal press self-stimulation rewarding experience in the layer V pyramidal neurons of the motor cortex and which is suggestive of increased usage of specific pathways to the cerebral cortex by pedal press behavior.

Changes in the dendritic spine density are important because they directly determine the size of the neuronal receptive field and influence the transmission of postsynaptic potentials to the cell body. Our neuromorphological findings on CA3 hippocampal and layer V motor cortical neurons add yet another component to the spectrum of alterations induced by rewarding experience. A regenerative and continuing mechanism for adaptive reorganization of the brain occurs because of the property of neuronal plasticity. The self-stimulation experience seems to be evoking this property strongly.

### *ICSS Induced Synaptic Plasticity*

Synaptic plasticity denotes long-term changes in synaptic potency resulting from transient changes in synaptic activity, the growth of new synapses, and the activation of silent synapses as well as changes in the efficacy of existing synapses. Synaptic junctions in the brain are very dynamic structures, which retain the ability to modify the number, nature and level of activity of synapses throughout the life span of animals. Alterations in synaptic number, size and shape have been reported following a number of manipulations such as electrical stimulation, long-term potentiation (LTP) and N-methyl-D-aspartate (NMDA) administration. Synaptic density in various regions of the central nervous system, including neocortex and hippocampus are altered in different conditions such as undernourishment, changes in hormonal levels, aging and Alzheimer's disease. Synaptic changes have also been noted following certain behavioural manipulations such as learning, imprinting, passive avoidance, visual and motor training and exposure to enriched environment (for more details refer Shankaranarayana Rao 1992, 1996, 1998).

Our previous studies have demonstrated a significant increase in the number of dendritic branching, intersections and dendritic spines in CA3 hippocampal and layer V motor cortical pyramidal neurons following self-stimulation of lateral hypothalamus (LH) and substantia nigra- ventral tegmental area (SN-VTA) for 10 days. Furthermore, SS also resulted in a significant increase in the concentration of noradrenaline, dopamine, glutamate and acetyl cholinesterase (AChE) activity in both hippocampus and motor cortex (Shankaranarayana Rao et al. 1998). Accordingly, we have investigated the alterations in the numerical density of synapses in the CA3 region of the hippocampus and the molecular layer of the motor cortex following self-stimulation rewarding behavioural experience.

Self-stimulation rewarding experience resulted in a significant increase in the number of synapses in CA3 region, which included moleculare, radiatum and lucidum layers of the hippocampus and mo-

lecular layer of the motor cortex (figure 3). Thus, self-stimulation rewarding experience induces synaptogenesis in hippocampal and motor cortical regions. Such changes in synaptic number could alter synaptic efficacy by altering the strength of the inputs to these neurons. Thus, an increase in synaptic number is well correlated with an increased dendritic arborization; spine densities as well as CA3 cell thickness following self-stimulation as shown in our earlier studies. Such changes may be responsible for the facilitation of acquisition and performance in operant and spatial learning tasks in SS experienced rats (Yoganarasimha et al. 1998).

Increased number of synapses following SS may activate the afferent pathways to the hippocampus and the motor cortex, which might lead to an induction of LTP. LTP is known to cause structural changes by strengthening the existing synapses or resulting in the formation of new synapses. Evidence for change in the number of synapses following LTP in the hippocampal region is well documented. In addition, the degree of potentiation is found to be associated with synaptic number during the maintenance of LTP in the rat dentate gyrus. The same mechanism might be responsible for an increased number of synapses observed in the motor cortex, since LTP was also demonstrated in the neocortex (Kirkwood et al. 1993). Recent studies have demonstrated the role of neurotransmitters in the regulation of the synaptic

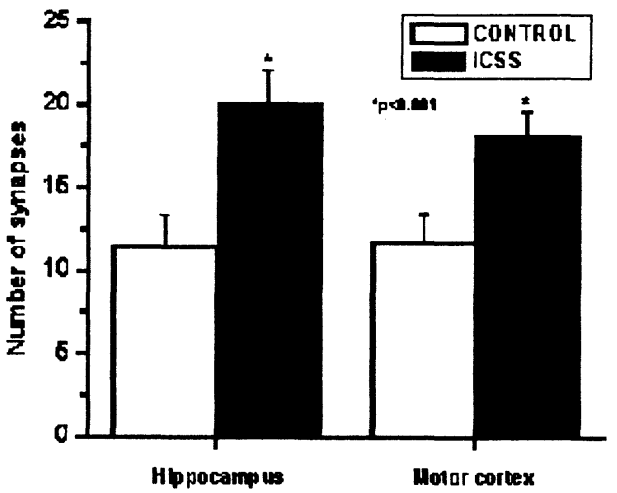


Figure 3 Number (mean ± SD) of synapses in CA3 hippocampal and motor cortical regions from control and ICSS experienced groups of rats. Note a significant (*p<0.001, two-way ANOVA) increase in the number of synapses in ICSS rats compared to control in both the regions (Shankaranarayana Rao 1996c).

density. The depletion of noradrenaline, serotonin and acetylcholine is found to decrease the synaptic number in rats (Matsukawa et al. 1995, 1997; Mazer et al. 1997). Our previous work has shown a significant increase in the levels of noradrenaline, dopamine, glutamate and AChE activity in hippocampus and motor cortex following SS experience (Shankaranarayana Rao et al. 1998b). Thus, altered concentration of these neurotransmitters may also play a role in regulating the synaptic activity in these regions.

The alterations in the synaptic density are considered as an important factor for the plastic changes in the nervous system to changing environmental conditions. Kleim et al (1996) have demonstrated an increase in the number of synapses in the motor cortex after motor learning and that this increase was dependent on the amount of training the animals receive. In addition, animals trained on a complex motor learning task showed an increase in synaptic number in Purkinje cells of cerebellar cortex and the learning dependent synaptic modification in the cerebellar cortex of the adult rats persist for at least four weeks. We have also observed long-lasting structural changes in the hippocampal and motor cortical neurons following self-stimulation experience in adult rats (Shankaranarayana Rao et al. 1997,1998c).

Eventhough there was an increase in the synaptic density in various learning paradigms, the magnitude of increase in synaptic number following self-stimulation was found to be higher. For example, in the adult rats subjected to brightness discrimination (Wenzel et al. 1980) and motor learning (Kleim et al. 1996) tasks, the synaptic density in hippocampus and motor cortex was increased by 40% and 18%, whereas in our study the number of synapses in these areas were increased by 76% and 56% , respectively. This suggests that the self-stimulation rewarding experience produces more pronounced effect than that produced by other forms of experiences.

In conclusion, the capacity of synapses to alter their structure and number in response to physiological usage may underlie or play a critical role in plastic neurobehavioral processes such as learning and memory. However, further research will determine which cellular processes are responsible for regulating the synaptic strength and number following self-stimulation experience in hippocampus and motor cortex.

### *ICSS Induced Structural Changes are Long-lasting*

Self-stimulation (SS) rewarding experience for 10 days results in long-lasting structural changes in CA3 hippocampal and layer V pyramidal neurons of the motor cortex, as there was an increase in the dendritic branching points and intersections in apical and basal dendrites in 30 days post- SS group (figure 4). However, the magnitude of increase in the number of branching points and intersections was found to be higher immediately after 10 days of self-stimulation experience (Shankaranarayana Rao et al. 1993, 1994) compared to the 30 and 60 days post-SS rats. When compared to 30 a 60 days post SS groups, the extent of increase was less in 60 days compared to 30 days.

Thus, our results clearly suggest that SS experience induces sustainable structural changes in hippocampal and motor cortical neurons. These results are in agreement with previous findings that the motor skills and the associated increase in synapse number with in the cerebellar cortex persist for at least 28 days after the cessation of training (Kleim et al. 1997). In addition, Bailey and Chen (1989) have showed an increased number of varicosities per sensory neurons associated with long-term sensitization of the gill withdrawal reflex in aplysia; also persist for atleast 3 weeks.

An increase in dendritic branching and intersections of hippocampal CA3 pyramidal neurons may be related to the learning experience associated with SS and this may enhance the capability of the rat in spatial cognitive functions (Yoganarasimha et al. 1998). An increase in the dendritic morphology in motor cortical neurons of the SS group may be related in part to the vigorous motor activity exhibited by rat during SS sessions. Such changes probably represent an increase in the afferent inputs to the motor cortical neurons from the sensory-motor pathways as well as the other neuronal circuitries involved in planning and execution of the motor activity.

Rutledge et al. (1974) have reported dendritic increase after long -term electrical stimulation in adult cat cortex and they suggested that increased use of specific pathways might produce post-synaptic growth of neurons. Previous studies have demonstrated SS induced increase in dendritic branching points, intersections, dendritic spines and synapses in CA3 hippocampal and layer V motor cortical pyramidal neurons ((Shankaranarayana Rao et al. 1993, 1994, 1998a,1999a,b). In addition, SS results in an increase

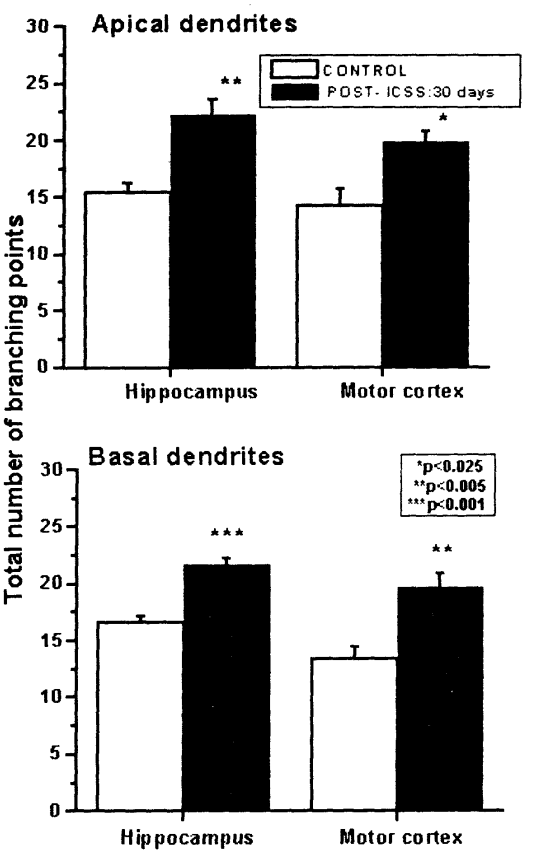


Figure 4 Number (mean ± SD) of branching points in apical and basal dendrites in CA3 hippocampal and layer V motor cortical pyramidal neurons from control and 30 days post-ICSS experienced groups of rats. Note a significant (*p<0.001, two-way ANOVA) increase in the number of branching points in 30 days post-ICSS experienced rats compared to control.

in the levels of glutamate, noradrenaline, dopamine and enhancement of AChE activity in the hippocampus and the motor cortex (Shankaranarayana Rao et al. 1998b). Furthermore, the prior SS experience is known to facilitate the acquisition of operant and spatial learning tasks in rats (Yoganarasimha et al. 1998). Such facilitation might be due to an increase in the dendritic arborization associated with neurochemical changes in the hippocampus.

In conclusion, a regenerative and continuing mechanism for adaptive reorganization of the brain occurs, because of the property of neuronal plasticity. The self-stimulation experience is evoking long-lasting structural changes and this may be due to the most intensive rewarding experience.

### *ICSS Induced Cytoskeletal Plasticity*

Experience-dependent structural plasticity is now a well documented phenomenon, however the central question about how functional demand operates at

the cellular level to influence the expression of cytoskeletal proteins, namely, neurofilaments, still remains to be answered. Neuronal growth, whether it is dendritic growth or the formation of new synapses are found to be accompanied by changes in the expression of cytoskeletal proteins, which are responsible for structural maintenance of the neurons. Neurofilaments are neuron-specific intermediate filament proteins, which constitute a major component of the neuronal cytoskeleton and play a critical role in determining shape and volume of neuronal processes including complex dendritic arborization and axonal caliber (Pant 1988). It has been suggested that the concentration of neurofilaments can serve as indicators for axonal development, neuronal plasticity, neuritic sprouting and nerve fiber regeneration (Doherty et al. 1984, Guth et al. 1989). Few studies have also correlated the expression of neurofilaments and neuronal maturation during development (Liu et al. 1994, Chaudari et al. 1996).

Several studies have shown that under normal conditions the neurofilaments in the neuronal perikarya and dendrites are non - phosphorylated while in axons they are phosphorylated (Sternberger & Sternberger 1983). Our recent studies have revealed an increased immuno-reactivity for phosphorylated and non-phosphorylated forms of neurofilaments in self-stimulation experienced rats in hippocampal and motor cortical pyramidal neurons (Shankaranarayana Rao 1996, (Shankaranarayana Rao et al. 2001b). The increased dendritic arborization after self-stimulation experience was associated with an enhanced expression of neurofilament proteins. These findings suggest a close relationship between the expression of cytoskeletal proteins and experience-dependent structural changes.

### *ICSS Induced Behavioral Plasticity*

A connection between ICSS and learning has been recognized since 1954, when Olds and Milner has shown that electrical stimulation of ICSS sites disrupted learning in experimental animals (Olds 1958, 1962). Since then neuroscientists have made attempts to link the brain reward and learning to understand the role of ICSS pathways in memory formation. Routtenberg (1978) suggested that rewarding brain stimulation applied in appropriate brain region and at the right moment in the learning process will alter the memory formation. In addition, he also speculated that the pathways of brain rewarding system might function as the neural substrate for memory consolidation.

ICSS rewarding experience seems to be a consistent way to facilitate learning and memory functions and has been shown to improve a wide variety of learned responses. Several studies indicate that ICSS can modulate learning and memory consolidation processes, such as aversive and appetitive classical conditioning (Coulombe & White 1980), sensory preconditioning (Coulombe and White, 1982), shuttle-box active avoidance learning (Aldavert-Vera et al. 1996, Segura-Torres et al. 1988, 1991) and can motivate treadmill running (Burgess et al. 1991) and weight-lifting exercise (Garner et al. 1991) in rats. Further, an improved retention of the one-trial appetitive learning task for water reward after hypothalamic self-stimulation has been demonstrated (Major & White, 1978). It has been suggested that the facilitatory effect of ICSS on these behavioral paradigms may be due to the general activation of neural systems and acceleration of memory consolidation process.

The post-training ICSS of the lateral hypothalamus facilitate the retention of a shuttle-box active avoidance task and improved retention of T-maze learning (Aldavert-Vera et al. 1996, Huston et al. 1977). In addition to the facilitatory effects of ICSS on learning and memory, various studies have demonstrated an increase in the locomotor activity in the running wheel (Schwarzberg et al. 1989). It has been shown recently that prior exposure to ICSS experience can induce facilitation of acquisition and better performance of operant and spatial learning tasks (table 5) (Yoganarasimha et al. 1996a,b, 1998). Such facilitatory effects observed may be due to an increased dendritic arborization and number of spines and synapses in hippocampus, accompanied with a rise in the levels of glutamate, dopamine, noradrenaline and acetylcholinesterase (AChE) activity have been reported following 10 days of ICSS-experience from lateral hypothalamus (LH) and ventral tegmental area (VTA) regions (Shankaranarayana Rao et al. 1993, 1994, 1998a-d, 1999a,b, 2001a). The involvement of other brain structures in bringing the facilitation cannot be ruled out. Because our previous studies have shown increased dendritic arborization in layer V neurons

of the motor cortex (Shankaranarayana Rao et al. 1993, 1994), as well as in the lateral hypothalamus and ventral tegmental neurons (Bindu & Desiraju 1990). Furthermore, ICSS experience ameliorates the behavioral deficits induced by fornix lesions (Yoganarasimha & Meti 1999). Interestingly, our recent experiments have showed that 10 days of self-stimulation rewarding experience can reverse the stress-induced behavioral deficits and cholinergic dysfunction (Ramkumar et al. 2001).

In summary, the facilitatory effects of ICSS on operant and spatial learning lead us to suggest that rewarding electrical stimulation could affect learning and memory by inducing an adequate activation of the neural system and the pathways that were able to affect a wide variety of conditioned responses. Further experiments are thus necessary to demonstrate a more specific effect of reinforcing brain stimulation on learning and memory process.

### *Electrophysiological studies*

The LH and SN-VTA brain regions are known to produce strongest expression of ICSS (Shankaranarayana Rao 1996). Accordingly, large number of electrophysiological experiments was aimed in characterizing these neurons and their interactions with the other brain regions. Single and multi unit activity studies have provided better understanding of the electrophysiological properties and characteristics of the LH and SN-VTA neurons. Since LH and SN-VTA are extensively connected to various limbic structures, which are also involved in ICSS like nucleus accumbens, olfactory tubercle, amygdala and hippocampus, several studies were carried out to evaluate the short-term effect of stimulation of limbic structures on the neuronal firing properties of LH and SN-VTA neurons (Bindu et al. 1992). The SS of MFB caused a significant increase in the firing rate of the cingulate cortex neurons, whereas, decreased the unit activity in the hippocampal neurons (Ito and Olds 1971, Ito 1972,1976).

Table 5 *Mean number of sessions to reach the criterion in operant and spatial learning tasks in self-stimulation experienced and control groups of rats*

| Group | Operant learning | Spatial learning (T-maze) |
|---|---|---|
| Normal control | 5.50 ± 0.54 | 6.33 ± 1.50 |
| Sham control | 5.83 ± 1.16 | 6.50 ± 1.64 |
| Self-stimulation | 3.00 ± 0.89** | 3.83 ± 0.73* |

Each value represents mean (± SD) data of 6 rats per group. *p<0.005; **p<0.001 (one-way ANOVA) (Yoganarasimha et al 1998).

Intracellular recordings revealed two types of neurons in the SN-VTA region, dopaminergic and non-dopaminergic (Grace & Bunney 1980, Yim & Mogenson 1980). Neurons with long duration action potentials, slow discharge rates and slow conduction velocity were characterized as dopaminergic neurons. Maeda and Mogenson (1980) have observed that electrical stimulation of preoptic area suppresses neuronal firing of both forms of SN- VTA neurons, whereas, nucleus accumbens stimulation caused more effect on dopaminergic neurons. The above studies reveal the electrophysiological characteristics of LH and SN-VTA neurons and the influence of other regions on the firing properties of these neurons.

Several studies have also provided the influence of various synaptic modulators on LH and SN-VTA neurons. Intravenous administration of apomorphine inhibits the dopamine cell firing of SN and haloperidol reverses this effect (Freeman et al. 1985). Bindu and Desiraju (1990) have studied systemic administration of dopaminergic and noradrenergic agonists and antagonists on LH and SN-VTA unit activity. They have showed that haloperidol increases firing of both LH and SN-VTA neurons, whereas, apomorphine reduces the firing in both the regions. It has also been shown that clonidine and yohimbine reduced firing rates in these neurons. In addition, electrical stimulation of LH increased the SN-VTA neuronal firing. On the contrary, stimulation of SN-VTA caused inhibition of firing responses in LH neurons (Bindu et al. 1992).

Future experiments need to be focused on evaluating the electrophysiological changes associated with ICSS in freely moving and behaving animals. Also it is necessary to characterize the rewarding neuronal properties and synaptic plasticity mechanisms using brain slice preparations.

## ICSS, an Animal Model for Drug Addiction

Drug addiction has afflicted mankind for centuries, yet the mechanisms by which particular drug lead to addiction, and the genetic factors that make some individuals particularly vulnerable to addic-

tion have remained elusive. From a clinical perspective, drug abuse continues to exact enormous human and financial costs on the society, yet all currently available treatments for drug addiction are notoriously ineffective. The search for a better understanding of the neurobiological mechanisms underlying the addictive actions of drugs of abuse and of the genetic factors that contribute to addiction should be given a high priority, as this should result in crucial advances in our ability to treat and prevent drug addiction.

From the basic neuroscience perspective, study of the neurobiology of drug addiction offers a novel opportunity to establish the biological basis of a complex and clinically relevant behavioral abnormality. Many prominent aspects of drug addiction in people can be clearly produced in laboratory animals, in striking contrast to most of other forms of neuropsychiatric illness. Thus, advances made in the study of drug addiction should provide important insights into the mechanisms underlying some of the psychiatric disorders.

In recent conceptualizations of drug reinforcement, the positive reinforcing properties of drugs have been thought to play an important role in drug dependence (Wikler 1973, Wise 1988). It is amply clear that animals and humans will readily self-administer drugs in the non-independent state and that drugs have powerful reinforcing properties in that animals will perform many different tasks to obtain drugs. The drugs that have positive reinforcing effects correspond well with the drugs that have high abuse potential in humans (see table 6). Much earlier work focused on operant paradigms in primates; however studies in the last few years have illustrated that many of these same paradigms can be utilized in rodent models and the new work in rodent models has provided a major benefit to studies focusing on the neurobiology of addiction (Koob & Bloom 1988, Koob & Goeders 1989).

Electrical self-stimulation of certain brain areas is rewarding for animals and humans as demonstrated by the fact that subjects will readily self-administer the stimulation (Olds & Milner 1954). The powerful nature of the reward effect produced by ICSS is indicated by the behavioral characteristics of the ICSS response, which include rapid learning and vigorous execution of the stimulation producing behavior (Gallistel 1983, Shankaranarayana Rao 1996). The high reward value of ICSS has led to the hypothesis that ICSS directly activates neuronal circuits that are activated by conventional reinforcers, like food, water and sex. In bypassing much of the input side of this neuronal circuit(s), ICSS provides a unique tool in neuropharmacological research to investigate the influence of various substances on reward and reinforcement processes.

Drugs of abuse decrease thresholds for ICSS, and there is a good correspondence between the ability of drugs to decrease ICSS thresholds and their abuse potential (Kornetsky & Esposito 1979). Many ICSS procedures have been developed over the years (Stellar & Stellar 1985), but an important methodological advance has been the development of procedures to provide a valid measure of reward threshold unconfounded by influences on motor and performance capability. Two ICSS procedures that have been used extensively to measure the changes in reward threshold produced by drugs are the rate-frequency, curve shift procedure and the discrete-trial, current-intensity procedure (Kornetsky & Esposito 1979, Markou & Koob 1993). These two procedures are widely used in ICSS research because they have been validated experimentally, but other valid ICSS procedures are also available (Markou & Koob 1993).

ICSS thresholds have been used to assess changes in systems mediating reward and reinforcement pro-

**Table 6** *Drugs that are self-administered by rats and monkeys*

| | |
|---|---|
| **Psychomotor Stimulants** | **Barbiturates** |
| Cocaine | Amobarbital |
| d-Amphetamine | Secobarbital |
| Methamphetamine | Pentobarbital |
| Phenmetrazine | Hexobarbital |
| Methylphenidate | |
| Diethylpropion | **Benzodiazepines** |
| | Chlordiazepoxide |
| **Opiates** | Diazepam |
| Morphine | |
| Meperidine | **Others** |
| Codeine | Ethanol |
| Pentazocine | Nicotine |
| Heroin | Phencyclidine |

cesses during the course of drug dependence. Acute administration of psychostimulant drugs lowers ICSS threshold (i.e., increases ICSS reward) (Kornetsky & Esposito 1979, Stellar & Stellar 1985) and withdrawal from chronic administration of these drugs elevates ICSS thresholds (i.e., decrease ICSS reward) (Kokkinidis & McCarter 1990, Leith & Barrett 1976, Markou & Koob 1991). Similar results have been observed with precipitated withdrawal in opiate-dependent rats (Schulteis et al. 1993). Rats trained in the discrete-trials threshold procedure showed dramatic increases in ICSS thresholds to naloxone injections that occurred in a dose-related manner and at doses below which obvious physical signs of opiate withdrawal were manifested.

The advantage of the ICSS paradigm as a model of drug effect on motivation and reward is that by directly stimulating the putative reward systems, one presumably bypasses the input side of the system and eliminates the nonspecific effects of consummatory behaviors, such as feeding, that can complicate data interpretation. Also, the behavioral threshold measure provided by ICSS procedures is easily quantifiable, because ICSS threshold estimates are very stable over periods of several months (Stellar & Stellar 1985). Another considerable advantage of the ICSS technique is the high reliability with which it predicts the abuse liability of drugs (Kornetsky & Esposito 1979).

## Significance of Animal Models of Addiction

The importance of animal models of drug addiction is the assumption that findings observed in the animal models will have relevance to the social problems of drug abuse and addiction in humans (Schuster & Thompson 1969). Certainly, animal models of drug addiction can predict abuse potential. However, the value of this animal model goes far beyond just the capacity to predict abuse potential. Animal models of drug addiction can provide a means of studying both the behavioral and biological basis of drug addiction. Factors involved in acquisition, maintenance, extinction and reinstatement of drug reinforcement can carefully be extracted in laboratory-controlled situations. The neurobiological mechanisms involved in the positive reinforcing effects of drugs and the negative reinforcing effects of drug abstinence can be elucidated. Perhaps even more importantly, the environmental, behavioral and neurobiological factors that contribute to individual differences in vulnerability to drug addiction can be explored with animal models.

Clearly, much remains to be explored about the neurobiological mechanisms of the unconditioned positive and negative motivational states and in particular the conditioned positive and negative motivational states associated with drug use and withdrawal. The study of the changes in the CNS that are associated with these homeostatic dysregulations may provide not only the key to drug dependence, but also the key to the etiology of psychopathologies associated with anxiety and affective abnormalities. The ICSS animal model described above provide a basis to begin such studies.

### *Neural Substrates of Addiction*

Mesolimbic dopaminergic neurons are thought to serve as a final common neural pathway for mediating reinforcement processes. There is a growing body of evidence suggests that dopamine is involved in the formation of associations between salient contextual stimuli and internal rewarding or aversive events. This evidence suggests that dopaminergic neuron activation aids the organism in learning to recognize stimuli associated with such events. Thus, mesolimbic dopaminergic neurons have an important function in the acquisition of behavior reinforced by natural rewards like food and sex, and drug stimuli. Accordingly, the role of mesolimbic dopamine system in drug addiction and reward mechanisms is discussed in detail (see below).

### *Mesolimbic Dopamine in Reward and Addiction*

Dopaminergic projection that originates in the ventral tegmental area (VTA) and projects to structures closely associated with the limbic system, most prominently the nucleus accumbens (nACB) region and the prefrontal cortex is particularly sensitive to electrical self-stimulation. Since, it is ubiquitously involved in the regulation of reward related behavior, this system considered to be the neurochemical substrate of reward (Wise 1996, Wise & Rompre 1989). Synaptic dopamine-mediated transmission in the nACB region is increased preferentially not only by natural rewards such as food, water and sex, but also by a variety of drugs abused by humans (Di Chiara & Imperato 1988,

Pontieri et al. 1995). Furthermore, it has been hypothesized that long-term adaptive changes within midbrain dopaminergic neurons take place following repeated drug administration and lead to a disruption or desensitization of neural mechanisms that mediate reward (Koob & Le Moal 1997, Wise 1996), while rendering these neurons more sensitive to other behavioral actions of drug stimuli (Robinson & Berridge 1993). Natural rewards, such as food and sex, as well as electrical brain-stimulation and many drugs of abuse increase the extracellular dopamine levels in the nACB (Wise & Rompre 1989, Di Chiara & Imperato 1988, Pontieri et al. 1995).

Both amphetamine and cocaine elevate extracellular dopamine concentrations in the terminal region of midbrain dopaminergic neurons, especially in the nACB (Di Chiara & Imperato 1998, Pontieri et al. 1995, Koob 1992). Extracellular dopamine levels are also reliably increased during self-administration of these drugs (Pettit & Justice 1989). However, psychostimulant-induced increases in synaptic dopamine in self-administering animals might involve factors other than only the direct pharmacological actions of these drugs. Experiments that examined the dopaminergic response to cocaine in self-administering rats with high time resolution suggest that the cocaine response be regulated by changes in extracellular dopamine levels in the nACB (Kiyatkin & Stein 1995, Wise et al. 1995, Peoples & West 1996). These observations extend earlier findings showing that selective destruction of midbrain dopamine neurons by 6-hydroxydopamine (6-OHDA) abolish cocaine self-administration (Roberts et al. 1980), and support the hypothesis that the mesolimbic dopaminergic system is crucial for psychostimulant reward.

Opiate drugs and opioid peptides can modulate mesolimbic dopamine activity. In vivo microdialysis data demonstrated that acute systemic or intracerebroventricular administration of $\mu$ or $\delta$ opioid receptor agonists increase dopamine release in the nACB, whereas k-opioid receptor agonists decrease dopamine release (Spanagel et al. 1990). Opioid receptor agonists increase extracellular dopamine levels within the nACB by disinhibiting GABA interneurons in the VTA (Johnson & North 1992). Activation of $\mu$-opioid receptors on GABAergic interneurons hyperpolarizes these interneurons and concomitantly disinhibits dopamine cell firing (Johnson & North 1992).

Several lines of evidence indicate that ethanol also activates the mesolimbic dopaminergic system. Alcohol injected intravenously increased firing of dopamine neurons in the VTA (Gessa et al. 1985), and acute administration of alcohol resulted in preferential release of dopamine from the nACB (Di Chiara & Imperato 1988, Imperato & Di Chiara 1986). Similar to opioid-induced stimulation of dopamine release, alcohol is thought to decrease the activity of GABAergic neurons in the VTA, which leads to a disinhibition of mesolimbic dopaminergic neurons (Kohl et al. 1998). Numerous pharmacological studies have investigated the role of midbrain dopaminergic neurons in alcohol reinforcement (Spanagel 1995). Rats will self-administer ethanol directly into the ventral tegmental cell body region of mesolimbic dopaminergic neurons (Gatto et al. 1994), and both D1 and D2 receptor antagonists administered either systemically or locally into the nACB decrease home-cage drinking and operant responses to alcohol (Samson et al. 1992, Hodge et al. 1997).

Repeated administration of many drugs of abuse can induce sensitized dopaminergic neuron responses; chronic drug administration can also lead to dopaminergic neuron dysfunction that becomes unmasked during withdrawal. Thus, marked inhibition of mesolimbic dopamine release seems to be a common feature of drug withdrawal in rats (Rosetti et al. 1992). Withdrawal from many drugs of abuse attenuates the rewarding effects of electrical brain stimulation, a phenomenon that has been linked to the negative emotional changes in withdrawal states (Koob & Le Moal 1997). As brain-stimulation reward is sensitive to disruption of mesolimbic dopamine transmission, withdrawal-associated reward deficits might be linked to impairments in mesolimbic dopamine-mediated transmission (Koob & Le Moal 1997).

The reinforcing and subjective, pleasurable effects of cocaine are thought to depend on blockade of the dopamine transporters (DAT) by cocaine, which results in increased synaptic availability of dopamine. A direct relationship between DAT blockade and self-report measures of 'high' was demonstrated in humans (Volkow et al. 1997a). The

degree and time course of DAT block by cocaine (as measured by PET) was correlated significantly with the temporal profile of cocaine euphoria and the binding kinetics of cocaine in the striatum of cocaine abusers. These results provide evidence for DAT blockade as a crucial mechanism in mediating the reinforcing and subjective effects of cocaine in humans.

Breiter et al. (1997) used functional magnetic resonance imaging (fMRI) in conjunction with behavioral ratings to investigate brain circuitries mediating cocaine-induced euphoria and craving in cocaine-dependent subjects. Following an infusion of cocaine, several brain regions, including the ventral tegmentum and basal forebrain, showed an immediate but transient activation that was correlated with reinforcement related ratings of 'rush'. Other regions, including the nACB and amygdala, showed sustained activation that was closely associated with incentive related measures of craving. Although some rush associated early activation was observed in the nACB, the temporal pattern of activation in this structure suggests that it may be involved in both reinforcement and incentive functions. Volkow et al. (1997b) has compared the responses of cocaine addicts and normal controls to intravenous methylphenidate, a drug that, like cocaine, increases synaptic dopamine levels. The effects of methylphenidate on both striatal dopamine release and subjective feelings of 'high' were significantly reduced in cocaine-dependent subjects. These findings are compatible with a decrease or desensitization in striatal dopamine function and a decrease in the reinforcing efficacy of psychostimulants, but contrast with the view that sensitization is a mechanism in human cocaine addiction.

Thus, there is now little doubt that midbrain dopamine has an essential role in the acquisition of drug-seeking behavior. Mesolimbic dopamine neurotransmission seem to have a crucial role in reinforcement maintained by drugs of abuse. Rather, it seems likely that dopaminergic neuron activation highlights important stimuli and functions as a learning signal. This function would also account for the growing evidence of dopaminergic neuron activation during reward expectation, and increased dopamine release associated with natural reinforcers under conditions of deprivation and novelty. This indicates that activation of mesolimbic dopaminergic neurons is necessary for intracranial self-stimulation (Garris et al. 1999).

Further studies on the biochemical and molecular basis of drug addiction are warranted, since they have several important clinical implications. A better understanding of neurobiological mechanisms underlying the addictive actions of drugs of abuse and of the genetic factors that contribute to drug addiction could lead to the development of pharmacological agents that prevent or reverse the effect of drugs on specific target neurons. Such drugs will be useful not only to treat physical abstinence syndromes, but also to reduce the craving for drugs of abuse. Their availability would represent a revolutionary step in our battle against drug addiction.

## ICSS, an Animal Model for Depression

ICSS paradigm provides an operational measure of anhedonia, a core feature of depression.Anhedonia in humans is defined as 'the markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day' (DSM-III-R). Because the anhedonia experienced by depressed patients suggests that these individuals might exhibit alterations in reward processes, the ICSS paradigm has been proposed as a model of depression. The ICSS paradigm requires that animals be prepared with intracranial electrodes aimed at specific brain sites. Because brief electrical stimulation of these sites is reinforcing, animals will work to electrically stimulate parts of their own brains. Both the rate response for ICSS and psychophysically defined threshold(s) for ICSS have been used as measures of the reward value of the stimulation (Markou & Koob 1992). Substantial evidence suggests that ICSS thresholds are reliable measures of reward (Markou & Koob 1992) that reflect the whole continuum from hedonia to anhedonia. Because it appears that ICSS acts directly on some of the same neuronal substrates that mediate the rewarding effects of natural reinforcers such as food, water and sex (Liebman & Cooper 1989), it is considered to be a valuable tool for the investigation of brain-reward systems.

The study of neural substrates of ICSS behavior following experiential or pharmacological manipulations promises to promote our understanding of reward mechanisms that seem to be altered in several psychiatric disorders, including depression and schizophrenia. Two manipulations have been used to produce an anhedonic state in animals, as operationally defined by decreases in ICSS response rates or elevations of thresholds, (i) exposure to uncontrollable stress (Zacharko et al. 1984) and (ii) withdrawal from long-term exposure to psychomotor stimulants (Kokkinidis et al. 1980, Markou & Koob, 1991). In the first of these procedures, mice exposed to either swim stress or uncontrollable footshock exhibit decrease in the reward value of ICSS (Valentino et al. 1990). These stress-induced alterations in ICSS behavior were reversed with repeated treatment with the tricyclic antidepressant, desmethylimipramine (Zacharko et al. 1984), indicating some predictive validity in terms of pharmacological isomorphism of the model. Potentially, the paradigm may have good etiological validity by inducing an anhedonic state through a mechanism that has been hypothesized to play a critical role in inducing depression in humans (Hammen & Cochran 1981).

The procedure for inducing an elevation in ICSS thresholds involves withdrawal from prolonged exposure to psychomotor stimulants, such as cocaine (Markou & Koob, 1991) or amphetamine (Kokkinidis et al. 1980). For example, when rats were withdrawn after being allowed to self-administer cocaine for prolonged periods of time, their thresholds for ICSS reward were elevated in proportion to the amount of cocaine consumed during the cocaine binge (Markou & Koob, 1991). Because the paradigm allowed rats to self-administer cocaine at doses and patterns that were reinforcing rather than aversive or stressful, the subsequent anhedonia cannot be attributed to the effects of uncontrollable stress but instead appears attributable to the pharmacological effects of cocaine. The use of the self-administration paradigm mimics humancocaine-bingeing episodes. Similar elevations in ICSS thresholds or decreases in response rates have also been observed following repeated treatment with experimenter-administered cocaine (Kokkinidis & McCarter 1990) or amphetamine (Kokkinidis et al. 1980), suggesting good reliability of the phenomenon. Tricyclics also appear to be effective in reversing the effects of withdrawal from amphetamine on ICSS (Kokkinidis et al. 1980). Although many more studies are needed, the evidence to date suggests that ICSS model has good construct and etiological validity and exhibits pharmacological isomorphism as a model for the drug induced anhedonia. Nevertheless, it is not known at this point what the relationship is between drug- induced and non-drug-induced depression in humans. Thus, the possible etiological validity of the ICSS paradigm as a model of non-drug-induced depression remains unclear. It has been argued that withdrawal from psychomotor stimulants induces most of the signs and symptoms typical of non-drug-induced depression in humans (Weddington et al. 1990). Future clinical and preclinical research needs to address this issue further.

Eventhough, ICSS model has good construct and etiological validity and exhibits drug induced anhedonia, many more models are required to address the complex issues in psychiatric disorders. Thus, there may be no perfect animal model, it is clear that each model has strengths and limitations that need to be recognized in order to use the model effectively in the investigation of psychiatric disorders. Therefore, multiple animal models are needed for each psychiatric disorder to allow investigation of the various aspects of the disease and to provide convergent validation of the research findings. Furthermore, each model is useful for a specific purpose, and the process of developing and validating animal models must work in concernt with the process of identifying reliable measures of the human phenomenology. However, one needs to be cautious in extrapolating animal data to humans particularly on brain functions.

**Acknowledgements**

One of the authors (B.S.S.Rao) acknowledges the Council for Scientific and Industrial Research (CSIR), New Delhi, for Research Associateship and the Indian National Science Academy (INSA), New Delhi, for INSA Young Scientist grant.

## References


Aldavert-Vera L, Segura-Torres P, Costa-Miserachs D and Morgado-Bernal I 1996 Shuttle-box memory facilitation by post-training self-stimulation differential effects in rats with high and low basic conditioning levels; *Behav. Neurosci.* 110 346-352

Amaral D G and Routtenberg A 1975 Locus coeruleus and intracranial self-stimulation: a Cautionary note; *Behav. Biol.* 13 331-338

Amalric M, Cline EJ, Martinez JL, Bloom FE and Koob GF 1987 Rewarding properties of b-endorphin as measured by conditioned place preference; *Psychopharmacology* 91 14-19

Andrews R J 1967 Intracranial self-stimulation in the chick; *Nature (Lond.)* 213 847- 848

Anis M A, Berry S C, Burton N R and Lodge D 1983 The dissociative anesthetics ketamine and phencyclidine selectively reduce excitation of central mammalian neurons by N-methyl-D-aspartate; *Br. J. Pharmacol.* 79 565-575

Atrens D M 1984 Self-stimulation and psychotropic drugs a methodological and conceptual critique; in *Animal Models in Psychopathology* pp 227-256 ed. N W Bond (Sydney: Academic Press)

Artola A and Singer W 1987 Long-term potentiation and NMDA receptors in the rat visual cortex; *Nature* 330 649-652

Bailey C H and Chen M 1989 Time course of structural changes at identified sensory neuons synapses during long-term sensitization in aplysia; *J. Neurosci.* 9 1774-1780

Balaban P M and Chase R 1989 Self-stimulation in snails; *Neurosci. Res. Commn.* 4 139-141

Baldwin B A and Parrott R F 1978 Intracranial electrical self-stimulation in pigs; *Proc. Physiol. Soc.* 12-13

Bals-Kubik R, Ableitner A, Herz A and Shippenberg T S 1993 Neuroanatomical sites mediating the motivational effects of opioids as mapped by the conditioned place preference paradigm in rats; *J. Pharmacol. Exp. Ther.* 264 489–495

Baxter B L, Gluckmen M I, Stein L and Scerni R A 1974 Self-injection of apomorphine in the rat: positive reinforcement by a dopamine receptor stimulant; *Pharmacol. Biochem. Behav.* 2 387-391

Ben-Ari Y and Repressa A 1992 Brief seizure episodes induce long-term potentiation and mossy fiber sprouting in the hippocampus; *Trends Neurosci.* 13 333-339

Belluzzi J D and Stein L 1977 Enkephalin may mediate euphoria and drive reduction reward; *Nature (Lond.)* 266 556-558

Beninato M and Spencer R F 1987 A cholinergic projection to the rat substantia nigra from the pedunculopontine tegmental nucleus; *Brain Res.* 412 167-174

Bindu P N 1990 *Neuropsychopharmacological investigations of the organization of the brain reward substrates of hypothalamus and midbrain*; PhD thesis Bangalore University, Bangalore

_____ and _____ 1990 Increase of dendritic branching of CA3 neurons of hippocampus and self-stimulation areas in subjects experienceing self-stimulation of lateral hypothalamus and substantia nigra-ventral tegmental area; *Brain Res.* 527 171-175

_____, Pandhari S R and Desiraju T 1992 Alterations of impulse discharges of units of lateral hypothalamus and ventral midbrain due to inter-regional stimulations or injections of catecholamine receptor ligands; in *Neurobiology: Principles of Design and Functions* pp155-164 ed. R Naresh Singh (New Delhi: Wiley Eastern)

Bishop M P Elder S T and Heith R G 1963 Intracranial self-stimulation in man; *Science* 140 394-396

Black W C and Cooper B R 1970 Reduction of electrically rewarded behaviour by interference with monoamine synthesis; *Physiol. Behav.* 5 1405

Boldry R C and Uretsky N J 1986 The importance of dopaminergic neurotransmission in the hypermotility response produced by adminstration of N-methyl-D-aspartic acid into the nucleus accumbens; *Neuropharmacol.* 27 375-381

Boyd E S, Gardner L C 1967 Effect of some brain lesions on intracranial self-stimulation in the rat; *Am. J. Physiol.* 213 1044-1052

Bozarth M A 1986 Neural basis of psychomotor stimulant and opiate reward evidence suggesting the involvement of a common dopaminergic system; *Behav. Brain Res.* 22 107-116

_____ 1987 Neuroanatomical boundaries of the reward-relevant opiate receptor field in the ventral tegmental area as mapped by the conditioned place preference method in rats; *Brain Res.* 414 77-84

_____ and Wise R A 1981 Intracranial self-administration of morphine in to the ventral tegmental area in rats; *Life Sci.* 28 551-555

_____ and _____ 1982 Localization of the reward- relevant opiate receptors; in *Problems of Drug Dependence* (NIDA Research Monograph No 41) pp 171- 177 ed. L S Harris (Washington: National Institute of Drug Abuse)

_____ and _____ 1984 Anatomically distinct opiate receptor fields mediate reward and physical dependance; *Science* 224 516-517

Brady J V 1971 Emotion revisited; *J. Psychiat. Res.* 8 363-384

Breiter H C, Gollub R L, Weisskoff R M, Kennedy D N, Makris N, Berke J D, Goodman J M, Kantor H L, Gastfriend D R, Riorden J P, Mathew R T, Rosen B R and Hyman S E 1997 Acute effects of cocaine on human brain activity and emotion; *Neuron* 19 591–611

Britt M D and Wise R A 1983 Ventral tegmental site of opiate reward antagonism by a hydrophilic opiate receptor blocker; *Brain Res.* 25 8105-108

Broekkamp C L, Phillips A G and Cools A R 1979 Facilitation of self-stimulation intracerebral microinjection of opoids into the ventral tegmental area; *Pharmacol. Biochem. Behav.* 11 289-295

Bruner A 1966 Facilitation of classical conditioning in rabbits by reinforcing brain stimulation; *Psychonomic Sci.* 6 211-212

Burgess M L, Davis J M, Borg T K and Buggy J 1991 Intracranial self-stimulation motivates treadmill running in rats; *J. Appl. Physiol.* 71 1593-1597

Campbell K A Evans G and Gallistel C R 1985 A microcomputer-based method for physiologically interpretable measurement of the rewarding efficacy of brain stimulation; *Physiol. Behav.* 35 395-403

Carey R J 1986 A examination of parkinsonian versus anhedonia contribution to self-stimulation impairment induced by dopamine dysfunction; *Behav. Brain Res.* 22 117- 125

Cassens G P and Mills A W 1973 Lithium and amphetamine: Opposite effects on threshold of intracranial reinforcement; *Psychopharmacologica* 30 283

Cazala P 1986 Self-stimulation behaviour can be elicited from various 'aversive' brain structures; *Behav. Brain Res.* 22 163-171

Chaudari A, Zangenchpour S, Matsubara J A and Cynader M S 1996 Differential expression of neurofilament protein in the visual system of the vervet monkey; *Brain Res.* 709 17-26

Christie M J, James L B and Beart P M 1985 An excitant amino acid projection from the medial prefrontal cortex to the anterior part of nucleus accumbens in rat; *J. Neurochem.* 454 477-482

Clarke P B S, Hommer D W Pert A and Skirboll L R 1987 Innervation of substantia nigra neurons by cholinergic afferent from pedunculopontine nucleus in the rat neuroanatomical and electrophysiological evidence; *Neuroscience* 23 1011-1019

Clavier R M and Routtenberg A 1976 Brain stem self-stimulation attenuated by lesions of medial forebrain bundle but not by lesions of locus coeruleus or the caudal ventral norepinephrine bundle; *Brain Res.* 101 251-271

_____ and Fibiger H C 1977 On the role of ascending catecholaminergic projections in intracranial self-stimulation of the substantia nigra; *Brain Res.* 131 271-286

_____ and Gerfen C R 1979 Self-stimulation of the sulcal prefrontal cortex in the rat direct evidence for ascending dopaminergic mediation; *Neurosci. Lett.* 12 183- 187

Collingridge G 1987 The role of NMDA receptors in learning and memory; *Nature* 330 604-606

Corbett D and Wise R A 1979 Intracranial self-stimulation in relation to the ascending noradrenergic fiber systems of the pontine tegmentum and caudal mid brain :A movable electrode mapping study; *Brain Res.* 177 423-436

_____ and Wise R A 1980 Intracranial self-stimulation in relation to the ascending noradrenergic fiber systems of the mid-brain: a movable electrode mapping study; *Brain Res.* 185 1-75

Coulombe D and White N 1980 The effect of post-training hypothalamic self-stimulation on aversive and appetite classical conditioning; *Physiol. Behav.* 25 267-272

_____ and _____ 1982 The effect of post training hypothalamic self-stimulation on sensory pre-conditioning in rats; *Can. J. Psychol.* 34 57-67

Crow T J 1969 Mode of enhancement of self-stimulation in rats by methamphetamine; *Nature* 224 709

_____ 1970 Enhancement by cocaine of intracranal self-stimulation in the rat; *Life Sci.* 9 375

_____ 1972 Catecholamine-containing neurones and electrical self-stimulation: a review of some data; *Psychol. Med.* 2 414-421

Culbertson J L, Kling J W and Berkley A 1966 Extinction responding following ICSS and food reinforcement; *Psychonomic Sci.* 5 127-128

Danysz W, Wroblewski J T and Costa E 1988 Learning impairment in rats by N-methyl-D-aspartate receptor antagonist; *Neuropharmacol.* 27 653-656

Delgado J M R, Roberts W W and Miller N E 1954 Learning motivated by electrical stimulation of the brain; *Am. J. Physiol.* 179 587-593

Di Chiara G and Imperato A 1988 Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats; *Proc. Natl. Acad. Sci., USA.* 85 5274–5278

Doherty P, Dickson J G, Flamigan T P and Walsh F S 1984 Quantitative evaluation of neurite outgrowth in cultures of human foetal brain and dorsal root ganglion cells using an enzyme-linked immunoadsorbant assay for human neurofilament proteins; *J. Neurochem.* 42 1116-1122

Domino E F and Olds M E 1968 Cholinergic inhibition of self-stimulation behaviour; *J. Pharmacol. Exper. Therap.* 164 202-211

_____ and _____ 1972 Effects of d-amphetamine scopolamine, chlordiazepoxide and diphenyl hydantoin on self-stimulation behaviour and brain acetyleholine; *Psychopharmacologia* 23 1

Dworkin SI, Guerin GF, Co C, Goeders NE and Smith JE 1988 Lack of an effect of 6-hydroxydopamine lesions of the nucleus accumbens on intravenous morphine self-administration; *Pharmacol. Biochem. Behav.* 30 1051–1057

Edmonds D E and Gallistel C R 1977 Reward versus performance in self-stimulation electrode specific effects of a-methyl-p-tyrosine on reward in the rat; *J. Comp. Physiol. Psychol.* 91 962-974

Engels J and Oreland L 1987 *Brain Reward Systems and Abuse*; pp 198 (New York: Raven Press)

Esposito R U and Kornetsky C 1977 Morphine lowering of self-stimulation threshold lack of tolerance with long term administration; *Science* 195 189

_____, Faulkner W and Kornetsky C 1979 Specification modulation of brain stimulation reward by haloperidol; *Pharmacol. Biochem. Behav.* 10 937-940

_____,Perry W and Kornetsky C 1980 Effects of d-amphetamine and naloxone on brain-stimulation reward; *Psychopharmacologica* **69** 187

_____,_____ and _____ 1981 Chlorpromazine and brain-stimulation reward potentiation of effects by naloxone; *Pharmacol. Biochem. Behav.* **15** 903

Ettenberg A and White N 1981 Pimozide attenuated conditioned taste preferences induced by self-stimulation in rats; *Pharmacol. Biochem. Behav.* **15** 915

_____,Pettit H O, Bloom F E and Koob G F 1982 Heroin and cocaine intravenous self-administration in rats: Mediation by separate neural systems; *Psychopharmacology* **78** 204-209

_____ 1989 Dopamine neuroleptics and reinforced behaviour; *Neurosci. Biobehav. Rev.* **13** 105.

Fenton H M and Liebmann J M 1982 Self-stimulation decrement patterns differentiate clonidine, baclofen and dopamine antagonists from drugs causing performance deficit; *Pharmacol. Biochem. Behav.* **17** 1207-1212

Fibiger H C, Lepiane F G Jukubovic A and Phillips A G 1987 The role of dopamine in intracranial self-stimulation of the ventral tegmental area; *J. Neurosci.* **7** 3888-3896

_____ and Philliphs A G 1981 Increased intracranial self-stimulation in rats and long-term administration of desipraine; *Science* **214** 683-685

Fouriezos G and Wise R A 1976 Pimozide-induced extinction of intracranial self-stimulation: response patterns rule out motor performance deficit; *Brain Res.* **103** 377-380

_____, Hansson P and Wise R A 1978 Neuroleptic-induced attenuation of brain-stimulation reward in rats; *J. Comp. Physiol. Psychol.* **92** 661-671

Fray P, Dunnett S, Iverson S, Bjorklund A and Stenevi V 1983 Nigral transplants reinnervating the dopamine-depleted neostriatum can sustain intracranial self-stimulation; *Science* **219** 416-419

Freeman A S, Melter L T and Bunney V S 1985 Firing properties of substantia nigra dopaminergic neurons in freely moving rats; *Life Sci.* **36** 1983-1994

Fujimoto K, Ikeguchi K and Yoshida M 1990 Decrease and recovery of choline acetyltransferase activity in medial thalamus and ventral tegmental area after destruction of pedunculopontine nucleus areas in the rat; *Neurosci. Res.* **9** 48-53

Gallistel C R 1973 Self-stimulation: The neurophysiology of reward and motivation; in *The Physiological Basis of Memory* pp 175-267 ed. J A Deutsh (New York: Academic Press)

_____,Shizgal P and Yeomans J S 1981 A portrait of the substrate for self-stimulation; *Psychological Rev.* **88** 228-273

_____,Boytim M, Gomita Y and Klebanoff L 1982 Does pimozide block the reinforcing effect of brain stimulation? *Pharmacol. Biochem. Behav.* **17** 769-781

_____, and Davis A J 1983 Affinity for the dopamine D2 receptor predicts neuroleptic potency in blocking the reinforcing effect of MFB stimulation; *Pharmacol. Biochem. Behav.* **19** 867-872

Gallistel C R 1983 Self-Stimulation in *The Physiological Basis of Memory* pp 73-77 ed. J A Deutsch (New York: Academic Press)

Ganong A H, Lanthorn T H and Cotman C W 1983 Kyneurenic acid inhibits synaptic and acidic aminoacid-induced responses in the rat hippocampaus and spinal cord; *Brain Res.* **273** 170-174

Garner R P, Terracio L, Borg T K and Buggy J 1991 Intracranial self-stimulation motivates weight-lifting exercise in rats; *J. Appl. Physiol.* **71** 1627-1631

Garrigues A M and Cazala P 1983 Central catecholame metabolism and hypothalamic self-stimulation behaviour in two inbred strains of mice; *Brain Res.* **265** 265-271

Garris P A, Kilpatrick M, Bunin M A, Michael D, Walker Q D and Wightman R M 1999 Dissociation of dopamine release in the nucleus accumbens from intracranial self-stimulation; *Nature* **398** 67–69

Gatto G J, McBride W J, Murphy J M, Lumeng L and Li T K 1994 Ethanol self-infusion into the ventral tegmental area by alcohol-preferring rats; *Alcohol* **11** 557–564

German D C and Bowden D M 1974 Catecholamine systems as the neural substrate for intracranial self-stimulation a hypothesis; *Brain Res.* **73** 381-419

Gessa G L, Muntoni F, Collu M, Vargiu L and Mereu G 1985 Low doses of ethanol activate dopaminergic neurons in the ventral tegmental area; *Brain Res.* **348** 201–203

Giorgnieff M F, Lefloc'h M L, West fall L C Glowinski J and Besson M J 1976 Nicotine effect of acetyl choline on the release of newly synthesized [$^3$H]-dopamine in rat striatal slices and cat caudate nucleus; *Brain Res.* **106** 117

Glimcher P W, Giovino A A, Margolin D H and Hoebel B G 1984 Endogenous opiate reward induced by an enkephalinase inhibitor thiorphan injected into the ventral midbrain; *Behav. Neurosci.* **98** 262-268

Goeders N E and Smith J E 1983 Cortical dopaminergic involvement in Cocaine reinforcement; *Science* **221** 773- 775

Gonzales R A and Weiss F 1998 Suppression of ethanol-reinforced behavior by naltrexone is associated with attenuation of the ethanol-induced increase in dialysate dopamine levels in the nucleus accumbens; *J. Neurosci.* **18** 10663–10671

Grace A A and Bunney B S 1980 Nigral dopamine neurons: Intracellular recording and identification with L-dopa injection and histofluorescence; *Science* **210** 654-656

Gratton A and Wise R A 1983 Brain stimulation reward in the lateral hypothalamic medial forebrain bundle mapping of boundaries and homogeniety; *Brain Res.* **274** 25-30

_____ and _____ 1985 Hypothalamic reward mechanisms: two first stage fiber populations with a cholinergic component; *Science* **227** 545-548

Greenough W T, Larson J R and Withers G S 1985 Effects of unilateral and bilateral training in a reaching task on dendritic branching of neurons of the rat somatosensory forelimb cortex; *Behav. Neural Biol.* **44** 310-314

Greenshaw A J, Sanger D J and Blackman D E 1983 Effects of d-amphetamine and of a-phenyl ethylamine on fixed internal responding maintained by self-regulated lateral hypothalamic stimulation in rats; *Pharmacol. Biochem. Behav.* 23 519

Guth L, Alberts R W, Barett C P and Donati E J 1989 Quantitative evaluation of axonal regeneration by immunochemical assay for neurofilament protein; *Exp. Neurol.* 100 83-97

Hammen C L and Cochran S D 1981Cognitive correlates of life stress and depression in college students; *J. Abnorm. Psychol.* 90 23-27

Heath R G 1963 Electrical self-stimulation of the brain in man; *Am. J. Psychiat.* 120 571-577

Heikkila R E Orlansky H and Cohen G 1975 Studies on the distinction between uptake inhibition and release of $^3$H- dopamine in rat brain tissue slices; *Biochem. Pharmacol.* 24 847

Hemby S E, Martin T J, Co C, Dworkin S I and Smith J E 1995 The effects of intravenous heroin administration on extracellular nucleus accumbens dopamine concentrations as determined by in vivo microdialysis; *J. Pharmacol. Exp. Ther.* 273 591–598

_____, Co C, Koves T R, Smith J E and Dworkin S I 1997 Differences in extracellular dopamine concentrations in the nucleus accumbens during response-dependent and response-independent cocaine administration in the rat; *Psychopharmacology* 133 7–16

Herberg L J and Williams S F 1983 Anti-conflict and depressant effects by GABA agonists and antagonists benzodiazepines and non-gabaergic anticonvulsants on self-stimulation and locomotor activity; *Pharmacol. Biochem. Behav.* 19 625-633

_____ and Rose I C 1989 Excitatory amino acid pathways mediate brain-stimulation reward ? *Psychopharmacol.* 96 S25

_____ and _____ 1990 Excitatory amino acid pathways in brain-stimulation reward; *Behav. Brain Res.* 39 230-239

Hodge C W, Samson H H and Chappelle A M 1997 Alcohol self-administration: further examination of the role of dopamine receptors in the nucleus accumbens; *Alcohol.Clin. Exp. Res.* 21 1083–1091

Hoebel B G, Hornandez L, McLeans S, Stanley B G, Aulissi E F, Glimcher P W and Margolin D 1982 Catecholamines, enkephalin and neurotensin in feeding and reward; in *The Neural Basis of Feeding and Reward* pp 465-478 eds B G Hoebel and D Novin (Bunswick M E: Haer Institute)

Howarth C I and Deutsch M 1982 Drive decay: the cause of fast "extinction" of habits learned from brain stimulations; *Science* 137 35-36

Hubner C B, Bain G T and Kornetsky C 1987 The combined effect of morphine and d-amphetamine on the threshold for brain-stimulation reward; *Pharmacol. Biochem. Behav.* 28 311

Huston J P, Mueller C C and Mondadari C 1997 Memory facilitation by post-trial hypothalamic stimulation and other reinforcers :A central theory of reinforcement; *Biobehav. Rev.* 1 143-150

Ichimaru Y, Moriyama M and Gomita Y 1983 Effects of antianxiety and antipsychotic drugs on DRL responding for brain stimulation; *Life Sci.* 32 437

Ikemoto S, McBride W J, Murphy J M, Lumeng L and Li T K 1997 6-OHDA-lesions of the nucleus accumbens disrupt the acquisition but not the maintenance of ethanol consumption in the alcohol-preferring P line of rats; *Alcohol. Clin. Exp. Res.* 21 1042–1046

Imperato A and Di Chiara G 1986 Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol; *J. Pharmacol. Exp. Ther.* 239 219–239

Ito M 1972 Excitability of medial forebrain bundle neurons during self-stimulation behavior; *J. Neurophysiol.* 35 652-664

_____ 1976 Mapping unit responses to reward stimulation; in *Brain stimulation reward* pp 89-95 eds A Wauquier and E T Rolls (Amsterdam: North-holland)

_____ and Olds J 1971 Unit activity during self-stimulation behavior; *J. Neurophysiol.* 34 263-273

Izenwassar S, Werling L L and Cox B M 1990 Comparison of the effects of cocaine and other inhibitors of dopamine uptake in rat striatum nucleus accumbens olfactory tubercle and medial prefrontal cortex; *Brain Res.* 520 303

Jenck F, Gratton A and Wise R A 1987 Opioid receptor subtypes associated with ventral tegmental facilitation of lateral hypothalamic brain stimulation reward; *Brain Res.* 423 34-38

Johnson S W and North R A 1992 Opioids excite dopamine neurons by hyperpolarization of local interneurons; *J. Neurosci.* 12 483–488

Jones T A and Schallert T 1994 Use-dependent growth of pyramidal neurons after neocortical damage; *J. Neurosci.* 14 2140-2152

Kalivas P W and Stewart J 1991 Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity; *Brain Res. Rev.* 16 223–244

Kent E W and Fedinets P 1976 Effects of GABA blockade on lateral hypothalamic self-stimulation; *Brain Res.* 104 628

Kirkwood A, Dudek S M, Gold J T, Aizenman C D and Bear M F 1993 Common forms of synaptic plasticity in the hippocampus and neocortex in vitro; *Science* 260 1518-1521

Kiyatkin E A and Stein E A 1995 Fluctuations in nucleus accumbens dopamine during cocaine self-administration behavior: an *in vivo* electrochemical study; *Neuroscience* 64 599–617

Kleim J A, Lussnig E, Schwartz E R and Greenough W T 1996 Synaptogenesis and FOS expression in the motor cortex of the adult rat after motor skill learning; *J. Neurosci.* 16 4529-4535

_____, Vij J, Ballard D H and Greenough W T 1997 Learning-dependent synaptic modifications in the cerebellar cortex of the adult tat persist for atleast 4 weeks; *J Neurosci* 15 717-721

Kofman O, McGlynn S M, Olmstead M C and Yeomans J S 1990 Differential effects of atropine, procaine and dopamine in the rat ventral tegmentum on lateral hypothalamic rewarding brain stimulation; *Behav.Brain Res.* 38 55

Kohl R R, Katner J S, Chernet E and McBride W J 1998 Ethanol and negative feedback regulation of mesolimbic dopamine release in rats; *Psychopharmacology* 139 79–85

Kokkinidis L and McCarter B D 1990 Postcocaine depression and sensitization of brain – stimulation reward analysis of reinforcement and performance effects; *Pharmacol. Biochem. Behav.* 36 463-471

_____, Zacharko R M and Predy P A 1980 Post-amphetamine depression of self-stimulation responding from the substantianigra reversal of tricyclic antidepressants; *Pharmacol. Biochem. Behav.* 13 379-383

Koob G F 1977 Incentive shifts in intracranial self- stimulation produced by different series of stimulus presentations; *Physiol. Behav.* 18 131-135

_____1992 Drugs of abuse: anatomy, pharmacology and function of reward pathways; *Trends Pharmacol. Sci.* 13 177–184

_____ and Bloom F E 1988 Cellular and molecular mechanisms of drug dependence; *Science* 242 715-723

_____and Goeders N 1989 Neuroanatomical substrates of drug self-administration; in *Neuropharmacological Basis of Reward* Vol 6 pp 214-264 eds J M Liebman and S J Cooper (New York: Oxford University)

_____ and Le Moal M 1997 Drug abuse: hedonic homeostatic dysregulation; *Science* 278 52–58

Kornetsky C and Bain G 1990 Brain-stimulation reward: A model for drug-induced euphoria; in *Modern Methods in Pharmacology* Vol 6 pp 211

_____and Esposito R U 1979 Euphorigenic drugs effects on reward pathways of the brain; *Fed. Proc.* 38 2473-2476

Kurumiya S and Nakajima S 1988 Dopamine D1 receptor in the nucleus accumbens involvement in the reinforcing effect of tegmental stimulation; *Brain Res.* 488 1-6

Lakshmana M K, Shankaranarayana Rao B S, Dhingra N K, Ravikumar R, Govindaiah, Ramachandra, Meti B L and Raju T R 1998 Chronic - deprenyl administration increases dendritic arborization in CA3 neurons of hippocampus and AChE activity in specific regions of the primate brain; *Brain Res.* 796 38-44

Lee K S, Schottler E, Oliver M and Lynch G 1980 Brief bursts of high frequency stimulation produce two types of structural changes in rat hippocampus; *J. Neurophysiol* . 44 247-258

Leith N J and Barret R J 1976 Amphetamine and the reward system evidence for tolerance and post-drug depression; *Psychopharmacologia* 46 19-25

Lestang I, Cardo B, Roy M T and Velley L 1985 Electrical self-stimulation deficits in the anterior and posterior parts of the medial forebrain bundle after ibotenic acid lesion of the middle lateral hypothalamus; *Neuroscience* 15 379-388

Liebman J M 1985 Anxiety anxiolytics and brain stimulation reinforcement; *Neurosci. Biobehav. Rev.* 9 75-86

_____ and Cooper S J 1989 *The Neuropharmacological Basis of Reward* (Oxford: Claredon Press)

Lilly J and Miller A M 1962 Operant conditioning of the bottlenose dolphine with electrical stimulation of the brain; *J. Comp. Physiol.* 55 73-79

Liu Y, Dyck R, Cynader, M The correlation between cortical neuron maturation and neurofilament phosphorylation: a developmental study of phosphorylated 200 KDa neurofilament protein in rat visual cortex; *Dev. Brain Res.* 81 151-161

Lorens S A and Sainati S M 1978 Naloxone blocks the excitatory effect of ethanol an chlordiazepoxide on lateral hypothalamic self-stimulation behaviour; *Life Sci.* 23 1359

Maeda H and Mogenson G J 1980 An electrophysiological study of inputs to neurons of the ventral tegmental area from the nucleus accumbens and medial preoptic-anterior hypothalamic areas; *Brain Res.* 197 365-377

Major R and White N 1978 Memory facilitation by self-stimulation reinforcement mediated by the nigro-neostriatal bundle; *Physiol. Behav.* 20 723-733

Maldonado R, Saiardi A, Valverde O, Samad T A, Roques B P and Borrelli E 1997 Absence of opiate rewarding effects in mice lacking dopamine D2 receptors; *Nature* 388 586–589

Mason P A Milner P M and Miousse R 1985 Preference paradigm provides better self-stimulation reward discrimination than a rate-dependent paradigm; *Behav. Neural Biol.* 44 521-529

Markou A and Koob G F 1991 Post-cocaine anhedonia: An animal model of cocaine withdrawal; *Neuropharmacology* 4 17-26

_____ and _____ 1992 Construct validity of self-stimulation threshold paradigm : Effects of reward and performance manipulations; *Physiol. Behav.* 51 111-119

_____ and _____ 1993 Intracranial self-stimulation thresholds as a measure of reward; in *Behavioral Neuroscience a Practical Approach* pp 93-115 ed. A Sahgal (New York: Oxford University Press)

_____, Hauger R L and Koob G F 1992 Desmethylimipraine attenuates cocaine withdrawal in rats; *Psychopharmacology* 109 305-314

Matsukawa M, Nakadate K and Okado N 1995 Synaptic loss following removal of serotonergic and/or noradrenergic fibers in the visual cortex of the developing and adult rat; *Soc. Neurosci. Abstr.* 21 182

_____, Ogawa M, Nakadate K, Maeshima T, Ichitani Y, Kawai N and Okado N 1997 Serotonin and acetylcholine are crucial to maintain hippocampal synapses and memory acquisition in rats; *Neurosci. Lett.* 230 13-16

Mazer C, Muneyyirci J, Taheny K, Raio N, Borella A and Whitaker-Azmitia P 1997 Serotonin depletion during synaptogenesis leads to decreased synaptic density and learning deficits in the adult rat: a possible model of neurodevelopment disorders with cognitive deficits; *Brain Res.* **760** 68-73

McCabe B J and Horn G 1988 Learning and memory regional changes in N-methy-D-aspartate receptors in chick brain after impriting; *Proc. NY Acad. Sci.* **85** 2849-2853

Mello N K and Mendelson J H 1979 Self-administration of an enkephalin analog by rhesus monkey; *Pharmacol. Biochem. Behav.* **9** 579-586

Merzenich M M and Kaas J H 1982 Reorganisation of mammalian somatosensory cortex following peripheral nerve injury; *Trends Neurosci.* **5** 434-436

Miserendino M J D and Coons E E 1989 Electrical stimulation of the medial prefrontal cortex supports both 'pure reward' and 'reward - escape' behavior in rats; *Brain Res.* **483** 226-232

Mitsuno K, Sasa M, Ishihara K, Ishikaw M and Kikudi H 1994 LTP of mossy-fiber stimulated potentials in CA3 neurons during learning in rats; *Physiol. Behav.* **55** 633-638

Mitchell M J, Nicolapu N M, Arbuthot G W and Yates C M 1982 Increase in dopamine metabolism are not a general feature of intracranial self-stimulation; *Life Sci.* **30** 1081-1085

Modell J G, Mountz J M, Glaser F B and Lee J Y 1993 Effect of haloperidol on measures of craving and impaired control in alcoholic subjects; *Alcohol. Clin. Exp. Res.* **17** 234-240

Mogenson G J 1964 Effects of sodium pentabarbital on brain self-stimulation; *J. Comp. Physiol. Psychol.* **58** 461

Mora F and Ferrer J M R 1986 Neurotransmitters pathways and circuits as the neural substrates on self-stimulation of the prefrontal cortex facts and speculations; *Behav. Brain Res.* **22** 127-140

Morris R G M, Anderson E, Lynch G S and Bandry M 1986 Selective impairment of learning and blockade of long-term potentation by an N-methyl-D-aspartate receptor antagonist AP5; *Nature* **319** 774-776

Nakahara D, Ozaki N, Kapoor V and Nagatsu T 1989 The effect of uptake inhibition on dopamine release from the nucleus accumbens of rats during self- or forced stimulation of the medial forebrain bundle: a microdialysis study; *Neurosci. Lett.* **104** 136

_____, Ozaki N, Miura J, Miura H and Nagatsu T 1989 Increased dopamine and serotonin metabolism in rat nucleus accumbens produced by intracranial self-stimulation of medial forebrain bundle as measured by invivo microdialysis; *Brain Res.* **495** 178-181

Nakajima S and McKenzie G M 1986 Reduction of the rewarding effect of brain stimulation by blockade of dopamine D1 receptor with SCH23390; *Pharmacol. Biochem. Behav.* **24** 919-923

Nassif S, Cardo B, Labersat F and Velley L 1985 Comparison of defects in electrical self-stimulation after ibotenic acid lesion of the lateral hypothalamus and the medial prefrontal cortex; *Brain Res.* **332** 247-257

Nazzaro J M and Gardner E L 1980 GABA antagonism lowers self-stimulation thresholds in ventral tegmental areas; *Brain Res.* **189** 279-283

Neil D B, Gaar L A, Clark A S and Britt M D 1982 "Rate-free" measures of self-stimulation and microinjections evidence towards a new concept of dopamine and reward; in *The Neural Basis of Feeding and Reward* pp 289- 298 eds B G Hoebel and D Novin (Brunswick M C: Heaer Institute)

Nestby P, Vanderschuren L J, De Vries T J, Mulder A H, Wardeh G, Hogenboom F and Schoffelmeer A N 1999 Unrestricted free-choice ethanol self-administration in rats causes long-term neuroadaptations in the nucleus accumbens and caudate putamen; *Psychopharmacology* **141** 307–314

Newman L M 1972 Effects of cholinergic agonists and antagonists on self-stimulation behaviour in rat; *J. Comp. Physiol. Psychol.* **79** 394-413

Niijima K and Yoshida M 1988 Activation of neuroencephalic dopamine neurons by chemical stimulation of the nucleus tegmenti pedunculopontinus pars compacta; *Brain Res.* **451** 163-171

Ods J 1958 Self-stimulation of the brain; *Science* **127** 315-327

_____ 1962 Hypothalamic substrate of reward; *Physiol. Rev.* **42** 554-604

_____ and Milner P 1954 Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain; *J. Comp. Physiol. Psychol.* **47** 419-429

_____ and Travis M P 1960 Effects of chlorpromazine, meprobamate, pentobarbital and morphine on self-stimulation; *J. Pharmacol. Exp. Ther.* **128** 397-404

Panskepp J, Gandelman R and Trowill J 1970 Modulation of hypothalamic self-stimulation and escape behaviour by chlordiazepoxide; *Physiol. Behav.* **5** 965

Pant H C 1988 Dephosphorylation of neurofilament proteins enhances their suceptibility to degradation by calpain; *Biochem. J.* **256** 665-668

Paxinos G and Wastson C 1982 *The Rat Brain in Stereotaxic Coordinates* (New York: Academic Press)

Peoples L L and West M O 1996 Phasic firing of single neurons in the rat nucleus accumbens correlated with the timing of intravenous cocaine self-administration; *J. Neurosci.* **16** 3459–3473

Pettit H O and Justice J B Jr. 1989 Dopamine in the nucleus accumbens during cocaine self-administration as studied by in vivo microdialysis; *Pharmacol. Biochem. Behav.* **34** 899–904

_____, Ettenberg A, Bloom F E and Koob G F 1984 Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats; *Psychopharmacology* **84** 167–173

Persson N 1962 Self-stimulation in the goat; *Acta. Physiol. Scand.* 55 276-285

Phillips A G, Blaha C D and Fibiger H C 1989 Neurochemical correlates of brain-stimulation reward measured by *ex vivo* and *in vivo* analysis; *Neurosci. Biobehav. Rev.*13 99

_____and Fibiger H C 1978 The role of dopamine in maintaining intracranial self-stimulation in the ventral tegmentum nucleus accumbens and medial prefrontal cortex; *Can. J. Psychol.* 32 58-66

_____ and LePaine F G 1980 Reinforcing effects of morphine microinjection into the ventral tegmental area; *Pharmacol. Biochem.Behav.* 12 965-968

_____ and _____ 1982 Reward produced by microinjection of D-Ala $^{2}$ Met$^{5}$ enkephalinamide into the ventral tegmental area; *Behav. Brain Res.* 5 225-229

_____ and _____ 1986 Effects of pimozide on positive and negative contrast with rewarding brain stimulation; *Pharmacol. Biochem. Behav.* 24 1577-1582

_____,Broekkemp C L and Fibiger H C 1983 Strategies for studying the neurochemical substrates of drug reinforcement in rodents; *Prog. Neuropsychopharmacol. Biol. Psychiat.* 7 585-590

_____, Jakurbovic A and Fibiger H C 1987 Increased invivo tyrosine hydroxylase activity in rat telencephalan produced by self-stimulation of ventral tegmental area; *Brain Res.* 402 109-116

_____, Lane R F and Blaha C D 1986 Inhibition of dopamine released by cholecystokinin: relevance to schizophrenia; *Trends Pharmacol. Sci.* 7 126-127

_____,Lepiene I G and Fibiger H C 1982 Effects of kainic acid lesions of the striatum on self-stimulation in the substantia nigra and ventral tegmental area; *Behav. Brain Res.* 5 297-310

_____, Vander Kooy D and Fibiger H C 1977 Maintenance of intracranial self-stimulation in hippocampus and olfactory bulb following regional depletion of noradrenaline; *Neurosci. Lett.* 4 77-84

Pontieri F E, Tanda G and Di Chiara G 1995 Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the "shell" as compared with the "core" of the rat nucleus accumbens; *Proc. Natl.Acad. Sci., USA* 92 12304–12308

Porrino L J and Coons E E 1980 Effects of GABA receptor blockade on stimulation-induced feeding and self-stimulation; *Pharmacol. Biochem Behav.* 12 125-130

Porter R W, Conrad D G and Brady J V 1959 Some neural and behavioural correlates of electrical self-stimulation of the limbic system; *J. Exp. Anal. Behav.* 2 43-55

Poschel B P H 1969 Mapping of rat brain for self-stimulation under monoamine oxidase blockade; *Physiol. Behav.* 4 325

_____ and Ninteman F W 1964 Excitatory antidepressant effects of monoamine oxidase inhibitors on the reward system of the brain; *Life Sci.* 13 903-910

_____ and _____ 1966 Psychotropic drug effects on self-stimulation of the brain a control for motor output; *Psychol. Rep.* 19 79

Poschel B P, Ninteman F W, McLean J R and Potoczak D 1974 Intracranial reward after 5,6-dihydroxytryptamine: further evidence for serotonin's inhibitory role; *Life Sci.* 15 1515-1522

Pradhan S N and Kamath K A 1973 Action and interaction of cholinergic agonists and antagonists on self-stimulation; *Arch. Internat. de Pharmacodynamics and Therapeutics* 196 321-329

Ramkumar K, Shankaranarayana Rao B S and Raju T R 2001 Self-stimulation rewarding experience reverses stress-induced behavioural deficits and cholinergic dysfunction (Submitted)

Rao D N 1985 *Study of cerebral electrical activity in brain-stimulation reward behaviour and its modulations by chemical and electrical stimulation;* Ph D thesis Bangalore University, Bangalore

Rao M S 1996 *Experimental study of neural transplants in behavioural recovery and patterns of survival and degeneration;* Ph D thesis NIMHANS Deemed University, Bangalore

Rassnick S, Stinus L and Koob G F 1993 The effects of 6-hydroxydopamine lesions of the nucleus accumbens and the mesolimbic dopamine system on oral self-administration of ethanol in the rat; *Brain Res.* 623 16–24

Redgrave P and Horrel R I 1976 Potentiation of central reward by local perfusion of acetylcholine and 5-hydrotryptamine; *Nature (Lond.)* 262 305-306

Roberts D C, Koob G F, Klonoff P and Fibiger H C 1980 Extinction and recovery of cocaine self-administration following 6-hydroxydopamine lesions of the nucleus accumbens; *Pharmacol. Biochem. Behav.* 12 781–787

Robinson T E and Berridge K C 1993 The neural basis of drug craving: an incentive-sensitization theory of addiction; *Brain Res. Rev.* 18 247–291

Rolls E T, Kelly P H and Shaw S G 1974 Noradrenaline, dopamine and brain-stimulation reward; *Pharmacol. Biochem. Behav.* 2 735-740

Rompre P P and Wise R 1988 A study of the interactions of pimozide, morphine and muscimol on brain stimulation reward behaviour: evidence for depolarization inactivation of A10– dopaminergic neurons; *Annals NY Acad. Sci.* 537 525-528

Rosenzweig M R 1966 Environmental Complexity, cerebal change and behaviour; *Am. Psychol.* 21 321-332

Rossetti Z L, Hmaidan Y and Gessa G L 1992 Marked inhibition of mesolimbic dopamine release: a common feature of ethanol, morphine, cocaine and amphetamine abstinence in rats; *Eur. J. Pharmacol.* 221 227–234

Routtenberg A 1978 The reward system of the brain; *Sci. Am.* 239 122-131

_____, Gardner E L and Huang Y H 1971 Self- stimulation pathways in monkey *Macaca mulatta*; *Exp. Neurol.* 33 213-234

Rutledge L T, Wright C, Duncan J 1974 Morphological changes in pyramidal cells of mammalian neocortex associated with increased use; *Exp. Neurol.* 44 209-228

Samson H H, Tolliver G A, Haraguchi M and Hodge C W 1992 Alcohol self-administration: role of mesolimbic dopamine; *Ann. New York Acad. Sci.* **654** 242j 253

Schaefer G J and Holtzman S G 1979 Free operant and auto- titration brain self-stimulation procedures in the rat: a comparison of drug effects; *Pharmacol. Biochem. Behav.* **10** 127-135

Schwartz R D, Uretsky N J and Bianchine J R 1980 The relationship between the stimulation of dopamine synthesis and release produced by amphetamine and high potassium in striatal slices; *J. Neurochem.* **35** 1120

Schwarzberg H, Roth N and Stunner I 1989 Increased locomotor activity of rats by self-stimulation in a running wheel; *Physiol. Behav.* **46** 767-769

Schulteis G, Carrera R, Markou A, Gold L H and Koob G F 1993 Motivation consequences of naloxone-precipitated opiate withdrawal a dose-response analysis; *Neurosci.* **19** 483-502

Schuster C R and Thompson T 1969 Self-administration and behavioral dependance on drugs; *Annu. Rev. Pharmacol. Toxicol.* **9** 483-502

Seeger T F, Carlson K R and Nazzaro J M 1981 Pentobarbital induces a naloxone-reversible decrease in mesolimbic self-stimulation threshold; *Pharmacol. Biochem. Behav.* **15** 583

Segura-Torres P, Capdevilla-Ortis L I, Marti-Nicoloviers M and Morgado-Bernal I 1988 Improvement of shuttle-box learning with pre- and post-training intracranial self-stimulation in rats; *Behav. Brain Res.* **29** 111-117

_____, Portell-Cortes I and Morgado-Bernal I 1991 Improvement of shuttle-box avoidance with post-training intracranial self-stimulation in rats a parametric study; *Behav. Brain Res.* **42** 161-167

Sem-Jacobson C W and Styri O G 1972 Electrical stimulation of the human brain pitfalls and results; in *Neurophysiological Studies in Man* pp 27-36 ed. G G Somien (Amsterdam: Excerpta Medica)

Shankaranarayana Rao B S 1992 *Plasticity in cerebral cortical neurons induced by self-stimulation rewarding behavioural experience;* MPhil Dissertation NIMHANS Deemed University, Bangalore

_____ 1998 Neuronal plasticity induced by self-stimulation rewarding behavioural experience Morphological Biochemical and Immunocytochemical evaluation; *NIMHANS J.* **16** 73-74

_____ and Desiraju T 1992 Plasticity of CA3 hippocampal and layer V motor cortical pyramidal neurons induced by self-stimulation experience; *Indian J. Physiol. Pharmacol.* **36** 42

_____ and _____ 1993 Plasticity in cerebral cortical neurons induced by self-stimulation rewarding behavioural experience; *NIMHANS J.* **11** 63-64

_____, _____, Meti B L and Raju T R 1994 Plasticity of hippocampal and motor cortical pyramidal neurons induced by self-stimulation experience; *Indian J. Physiol. Pharmacol.* **38** 23-28

_____, _____ and Raju T R 1993 Neuronal plasticity induced by self-stimulation rewarding experience in rats - a study on alteration in dendritic branching in pyramidal neurons of hippocampus and motor cortex; *Brain Res.* **627** 216-224

_____, Raju T R and Meti B L 1993 Noradrenaline, dopamine and serotonin levels in hippocampus and motor cortex after self-stimulation experience; *Indian J. Physiol. Pharmacol.* **37** 73-74

_____, _____ and _____ 1994 Effect of self-stimulation rewarding experience on the dendritic spine density in CA3 hippocampal and layer V motor cortical pyramidal neurons; *Indian J. Physiol. Pharmacol.* **38** 42

_____, _____ and _____ 1995a Self-stimulation rewarding experience induced alterations in dendritic spine density in CA3 hippocampal and layer V motor cortical pyramidal neurons; *Indian J. Physiol . Pharmacol.* **39** 9

_____, _____ and _____ 1995b Self-stimulation experience induced alterations in the levels of glutamate, GABA and AChE activity in hippocampus and motor cortex; *Indian J. Physiol. Pharmacol.* **39** 22

_____, _____ and _____ 1996a Neuronal plasticity induced by self-stimulation rewarding experience in hippocampus and motor cortex - morphological and neurochemical evaluation; *Proc. Int. Symp. Mol. Struct. Dev. Func. Organisms* 36-37

_____, _____ and _____ 1996b Synaptic plasticity in hippocampus and motor cortex induced by self-stimulation rewarding experience; *Proc. 38th Annual Conf. Indian Soc. Aerospace Med.* **38** 47-48

_____ 1996c *Neuronal plasticity induced by self-stimulation rewarding behavioural experience: Morphological Biochemical and immunocytochemical evaluation;* Ph D thesis NIMHANS Deemed University, Bangalore

_____, Raju T R and Meti B L 1997 Long-lasting structural changes in CA3 hippocampal and layer V motor cortical pyramidal neurons associated with self-stimulation rewarding experience- A quantitative Golgi Study; *Proc. 84th Indian Sci. Cong. Part III* **84** 60

_____, Shahani N Raju T R and Meti B L 1997 Alterations in the expression of neurofilament proteins in self-stimulation experienced rats; *Proc. 15th Annual Conf. Indian Acad. Neurosci.* **15** 21

_____, Meti B L and Raju T R 1998 Neuronal plasticity-A unique property for Neurorehabilitation In *Neurorehabilitation: Principles and Practice* pp 36-49 eds A B Taly, K P S Nair and T Murali (Bangalore: National Institute of Mental Health and Neurosciences)

_____, Raju T R and Meti B L 1998a Alterations in the density of excrescences in CA3 neurons of hippocampus in rats subjected to self-stimulation experience; *Brain Res.* **804** 320-324

_____, _____ and _____ 1998b Self-stimulation of lateral hypothalamus and ventral tegmentum increases the levels of noradrenaline, dopamine, glutamate and AChE activity but not 5-hydroxytryptamine and GABA levels in hippocampus and motor cortex; *Neurochem. Res.* **23** 1053-1059

_____, _____ and _____ 1998c Long-lasting structural changes in CA3 hippocampal and layer V motor cortical pyramidal neurons associated with self-stimulation - a quantitative Golgi study; *Brain Res. Bull.* **47** 95-101

_____, _____ and _____ 1998d Self-stimulation rewarding experience induced alterations in the levels of biogenic amines, aminoacids and AChE activity in hippocampus and motor cortex; *J. Neurochem.* **70** Suppl 2 S32A

_____, _____ and _____ 1999a Self-stimulation rewarding experience induced alterations in dendritic spine density in CA3 hippocampal and layer V motor cortical pyramidal neurons; *Neuroscience* **89** 1067-1077

_____, _____ and _____ 1999b Increased numerical density of synapses in CA3 region of hippocampus and molecular layer of motor cortex after self-stimulation rewarding experience; *Neuroscience* **91** 799-803

_____, Lakshmana M K, Meti B L and Raju T R 1999 Chronic (-)deprenyl administration alters dendritic morphology of layer III pyramidal neurons in the prefrontal cortex of adult Bonnett monkeys; *Brain Res.* **821** 218-223

_____, Meti B L and Raju T R 2001a Neuronal plasticity-A unique property for Neurorehabilitation; in *Neurorehabilitation Principles and Practice* 2nd edn eds A B Taly K P S Nair and T Murali pp 27-37 (New Delhi: Ahuja Book Company Pvt. Ltd.)

_____, _____ and _____ 2001b Self-Stimulation rewarding experience induced alterations in the expression of neurofilament proteins in hippocampus and motor cortex (Submitted)

Shippenberg T S, Bals-Kubik R and Herz A 1993 Examination of the neurochemical substrates mediating the motivational effects of opioids: role of the mesolimbic dopamine system and D-1 vs. D-2 dopamine receptors; *J. Pharmacol. Exp. Ther.* **265** 53–59

Smith J E and Lane J D 1983 *The Neurobiology of Opiate Reward Processes* pp 415 (Amsterdam: Elsevier)

_____ and Dworkin S I 1986 Neurobiological substrates of drug self-administration In *Opiate Receptor Subtypes and Brain Function* NIDA Research Monograph No 71 pp 127-145 eds R M Brown, D H Clout and D P Friedman (Washington: National Institute on Drug Abuse)

Singh J 1990 *Study of effects of synaptic transmission modulators of classical neurotransmitters and neuropeptides on the brain reward system and other basic behaviours;* Ph D thesis Bangalore University, Bangalore

_____, Desiraju T, Nagaraja T N and Raju T R 1994 Facilitation of self-stimulation of ventral tegmentum by microinjection of opioid receptor subtype agonists; *Physiol. Behav.* **55** 627-31

_____, Desiraju T and Raju T R 1996 Dose-response functions of apomorphine, SKF 38393, LY 171555, haloperidol and clonidine on the self-stimulation evoked from lateral hypothalamus and ventral tegmentum; *Indian J. Physiol. Pharmacol.* **40** 15-22

_____, _____ and _____ 1997a Cholinergic and GABAergic modulation of self-stimulation of lateral hypothalamus and ventral tegmentum effects of carbachol, atropine, bicuculline and picrotoxin; *Physiol. Behav.* 61 411-418

_____, _____ and _____ 1997b Dopamine receptor subtypes involvement in nucleus accumbens and ventral tegmentum but not in medial prefrontal cortex on self-stimulation of lateral hypothalamus and ventral mesencephalon; *Behav. Brain Res.* **86** 171-179

Spragg S D S 1940 Morphine addiction in chimpanzees; *Comp. Psychol. Monographs* **15** 11-32

Spanagel R 1995 Modulation of drug-induced sensitization processes by endogenous opioid systems; *Behav. Brain Res.* **70** 37–49

_____ and Zieglgansberger W 1997 Anti-craving compounds for ethanol: new pharmacological tools to study addictive processes; *Trends Pharmacol. Sci.* 18 54–59

_____, Herz A and Shippenberg T S 1990 The effects of opioid peptides on dopamine release in the nucleus accumbens: an in vivo microdialysis study; *J. Neurochem.* 55 1734–1740

Stark P and Boyd E S 1963 Effects of cholinergic drugs on hypothalamic self-stimulation response rates in dogs; *Amer. J. Physiol.* 205 745-748

Stein L 1962 Effects and interactions of imipramine, chlorpromazine, reserpine and amphetamine on self-stimulation possible neurophysiological basis of depression; in *Recent Advances in Biological Psychiatry* (Vol 4) pp 288-308 ed. J Wortis (New York: Plenum Press)

_____ 1964 Self-stimulation of the brain and the central stimulant action of amphetamine; *Fed. Proc.* 23 836-850

_____ 1975 Norepinephrine reward pathways; role in self-stimulation memory consolidation and schizophrenia; in *Nebraska Symposium on Motivation* pp 113-159 eds J Cole and T Sondregger (Lincoln N E: University of Nebraska Press)

_____ 1985 Effects of intracerebral self-stimulation on brain opioid peptides; *Peptides* 6 67-73

_____ and Belluzzi J D 1979 Brain endorphins possible role in reward and memory formation; *Fed. Proc.* 28 2468- 2472

_____ and _____ 1987 Reward transmitters and drugs of abuse; in *Brain Reward Systems and Abuse* eds Enger J and Oreland L pp 19-33 New York Raven Press

_____ and Wise C D 1969 Release of norepinephrine from hypothalamus and amygdala by rewarding median forebrain bundle stimulation and amphetamine; *J. Comp. Physiol. Psychol.* **67** 189-198

Sternberger L A and Sternberger N H 1983 Monoclonal antibodies distinguish phosphorylated and non-phosphorylated forms of neurofilaments in situ; *Proc. Natl. Acad. Sci. USA* **80** 6126-6130

Stellar J R and Stellar E 1985 *The Neurobiology of Motivation and Reward* pp249 (New York: Springer-Verlag)

_____, Kelley A E and Corbett D 1983 Effects of peripheral and central dopamine blockade on lateral hypothalamic self-stimulation Evidence for both reward and motor deficits; *Pharmacol. Biochem. Behav.* **18** 433-442

Takeichi T, Kurumiya S, Umemoto M and Olds M E 1985 Role of catecholamine terminals and intrinsic neurons of the ventral tegmentum in self-stimulation investigated in neonatally dopamine-depleted rats; *Pharmacol. Biochem. Behav.* **24** 1101-1109

Tokumo H, Morizumi T, Kudo M and Nakamura Y 1988 A morphological evidence for monosynaptic projections from the nucleus tegmenti pedunculopontinus pars compacta to nigrostriatal projection neurons; *Neurosci. Lett.* **85** 1-4

Uretsky N J and Snodgrass S R 1977 Studies on the mechanism of stimulation of dopamine synthesis by amphetamine in striatal slices; *J. Pharmacol. Exp. Ther.* 202 565

Valentino D A, Dufresne R L and Riccitelli A J 1990 Effects of a single inescapable swim on long-term brain stimulation reward thresholds; *Physiol. Behav.* **48** 215-219

Van Heuven-Nolsen D, Van Wolfswinkel L, and Van Ree J M and Versteeg D H G 1983 Electrical stimulation of the ventral tegmental area and catecholamine metabolism in discrete regions of the rat brain; *Brain Res.* **268** 362-366

Vander Kooy D, Fibiger H C and Phillips A G 1977 Monoamine involvement in hippocampal self-stimulation; *Brain Res.* **136** 119-130

Van Ree J M and Ramsey N 1987 The dopamine hypothesis of opiate reward challenged; *Eur. J. Pharmacol.* **134** 239–243

_____ and de Wied D 1980 Involvement of neurohypophyseal peptides in drug-mediated adaptive responses; *Pharmacol. Biochem. Behav.* **13** (Suppl 1) 257-263

Velley L 1986 The role of intrinsic neurons in lateral hypothalamic self-stimulation; *Behav. Brain Res.* **22** 141- 152

_____, Chamniade C, Roy M P Kempf E and Cardo B 1983 Intrinsic neurons are involved in lateral hypothamalmic self-stimulation; *Brain Res.* **268** 79-86

_____, Verney C, Kempe E and Berger B 1988 Opposite effects of ibotenic acid and 6-hydroxy dopamine lesions of the lateral hypothalamus on intracranial self-stimulation and stimulation-induced locomotion; *Neuroscience* **25** 595-603

Volkow N D, Wang G J, Fischman M W, Foltin R W, Fowler J S, Abumrad N N, Vitkun S, Logan J, Gatley S J, Pappas N, Hitzemann R and Shea C E. 1997a Relationship between subjective effects of cocaine and dopamine transporter occupancy; *Nature* **386** 827–830

_____, _____, Fowler J S, Logan J, Gatley S J, Hitzemann R, Chen A D, Dewey S L and Pappas N 1997b Decreased striatal dopaminergic responsiveness in detoxified cocaine-dependent subjects; *Nature* **386** 830–833

Walsh R N, Bedtz-Olsen O E, Penny J E and Cummins RA 1972 The effects of environmental complexity on the histology of the rat hippocampus; *J. Comp. Neurol.* **137** 361- 366

Wauquier A 1979 Neuroleptics and brain stimulation behavior; *Int. Rev. Neurobiol.* **21** 335-403

_____ and Niemegeers C J E 1974 Intracranial self-stimulation in rats as a function of various stimulus parameters IV. The influence of amphetamine on medial forebrain bundle stimulation with monopolar electrodes; *Psychopharmacologica* **34** 265

_____ and Rolls E 1976 *Brain Reward System* (New York: Elsevier)

Weddington W W, Brown B S and Haertzen C A 1990 Changes in mood craving and sleep during short-term abstinence reported by male cocaine addicts; *Arch. Gen. Psychiat.* **47** 861-868

Wenzel S, Kawerer E, Kirsche W, Metthies H and Wenzel M 1980 Electron microscopic and morphometric studies on synaptic plasticity in the hippocampus of the rat following conditioning; *J. Hirnforsch.* **21** 647-654

Wetzel M C and King J E 1966 Self-stimulation with monophasic current in the rock squirrel and rats; *Psychonomic Sci.* **6** 7-8

Wilkinson H A and Peele T L 1963 Intracranial self-stimulation in cats; *J. Comp. Neurol.* **121** 425-440

Wikler A 1973 Dynamics of drug dependance: implications of a conditioning theory of research and treatment; *Arch. Gen. Psychiat.* **28** 611-616

Withers G S and Greenough W T 1989 Reach training selectively alters dendritic branching in subpopulation of layer II-III pyramids in rat motor-somatosensory forelimb cortex; *Neuropsychologia* **27** 61-69

Wise R A 1980a Action of drugs of abuse on brain reward system; *Pharmacol. Biochem. Behav.* **13** (Suppl 1) 213-223

_____ 1980b The dopamine synapse and the notion of 'pleasure centres' in the brain; *Trends Neurosci.* 3 91-95

_____ 1981 Intracranial self-stimulation mapping against the lateral boundaries of the dopaminergic cells of substantia nigra; *Brain Res.* **213** 190-194

_____ 1987 The role of reward pathways in the development of drug dependence; *Pharmacol. Therap.* **35** 227-265

_____ 1988 The neurobiology of craving implications for the understanding and tratment of addiction; *J. Abnorm. Psychol.* **97** 118-132

_____ 1996 Neurobiology of addiction; *Curr. Opin. Neurobiol.* 6 243–251

_____ and Rompre P P 1989 Brain dopamine reward; *Ann. Rev. Psychol.* 40 191-225.

_____ and Bozarth M A 1984 Brain reward circuity four circuit element "wired" in apparent series; *Brain Res. Bull.* 12 203-209

_____, Newton P, Leeb K, Burnette B, Pocock D and Justice JB Jr 1995 Fluctuations in nucleus accumbens dopamine concentration during intravenous cocaine self-administration in rats; *Psychopharmacology* 120 10–20

Wong E H F, Kemp J A, Priestley T Knight A R Woodruff G N and Inversen L L 1988 The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist; *Proc. Natl. Acad. Sci., USA* 83 7104-7108

Woolf N J, Harrison N J and Buchwald J S 1990 Cholinergic neurons of the feline pontomesencephalon II Ascending anatomical projections; *Brain Res.* 520 55-72

_____ 1991 Cholinergic systems in mammalian brain and spinal cord; *Prog. Neurobiol.* 37 475-524

Yeomans J S, Mathur A and Tampakeras 1993 Rewarding brain stimulation: Role of tegmental cholinergic neurons that activate dopamine neurons; *Behav. Neurosci.* 107 899-910

_____, Kofman O and McFarlane V 1985 Cholinergic involvement in lateral hypothalamic rewarding brain stimulation; *Brain Res.* 329 19-26

Yim C Y and Mogenson G J 1980 Electrophysiological studies of neurons in the ventral tegmental area of Tsai. *Brain Res.* 181 301-313

Yoganarasimha D, Shankaranarayana Rao B S, Raju T R and Meti B L 1996a Self-stimulation rewarding experience facilitates the operant and spatial learning; *Proc. 38th Annual Conf. Indian Soc. Aerospace Med.* 38 48-49

_____, _____, _____ and _____ 1996b Effect of self-stimulation rewarding experience on the acquisition and performance of operant and spatial learning in rats; *Indian J. Physiol. Pharmacol.* 40 15

_____, _____, _____ and _____ 1998 Facilitation of acquisition and performance of operant and spatial learning tasks in self-stimulation experienced rats; *Behav. Neurosci.* 112 725-729

_____ and Meti BL 1999 Amelioration of fornix lesion induced learning deficits by self-stimulation rewarding experience; *Brain Res.* 845 246-251

Zacharko R M, Bowers W J, Kelly M S and Anisman H 1984 Prevention of stressor-induced disturbances of self-stimulation by desmethylimipraine; *Brain Res.* 321 175-179

Zarevics P and Setler P E 1979 Simultaneous rate-independent and rate-dependent assessment of intracranial self-stimulation:evidence for the direct involvement of dopamine in brain reinforcement mechanisms; *Brain Res.* 169 499-512

_____ and _____1981 Effects of gabaeric drugs on brain stimulation reward as assessed by a threshold method; *Brain Res.* 215 201-209