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The Effects of a Mixed Dopamine D2/D3 Agonist and Antagonist on
Ethanol Intake Within a Limited and a Continuous Access Paradigm.

Lana Marie Pratt

A Thesis

in

The Department

of

Psychology

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ABSTRACT

The Effects of a Mixed Dopamine D2/D3 Agonist and Antagonist on Ethanol
Intake Within a Limited and a Continuous Access Paradigm.

Lana Marie Pratt

The effects of the mixed dopamine D2/D3 agonist, quinpirole and antagonist,
raclopride on ethanol consumption were examined within both a limited and
a continuous access paradigm. The goals of these studies were to determine if
decreases in ethanol intake after administration of the dopaminergic agents
were the result of the type of paradigm employed, reductions of DA-mediated
reinforcement or rather to non-specific drug effects, such as locomotor
suppression. Experiment 1 demonstrated that quinpirole (0.1 mg/kg) failed to
alter ethanol intake in rats exposed to a 1hr-limited access period. Raclopride-
treated (0.5 mg/kg) rats significantly decreased their ethanol intake but were
ataxic for the entire 1-hr limited access period. These results suggested that
manipulations of the D2/D3 receptors at the dose tested had non-specific
effects on the consumption of ethanol. Experiment 2 assessed whether the
effects obtained within a limited access paradigm were comparable to a 22-hr
continuous access paradigm. Similar, to Experiment 1, quinpirole (0.1 mg/kg)
failed to attenuate ethanol consumption within a continuous access
paradigm. Contrary to Experiment 1, raclopride (0.5 mg/kg) significantly
increased ethanol intake during the treatment and post-treatment periods.
Taken together, the findings of Experiment 1 and 2 demonstrated that the
D2/D3 agents employed did not produce reductions in ethanol intake specific
to ethanol reinforcement. Further, the raclopride induced increase in ethanol
intake in Experiment 2 is contradictory to previous reports that systemic
injections of DA antagonists produce reductions in ethanol intake. The

results suggested that the D2/D3 receptors are not likely to be the primary mediators of ethanol intake.

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To Tristan, you were and are my inspiration.

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INTRODUCTION

The voluntary self-administration of alcohol, a behavior common to most societies, has frequently been associated with times of celebration, relaxation and enjoyment (Altman, Everitt, Glautier, Markou, Nutt et al., 1996). Unfortunately, for approximately 6 % to 14% of the population, alcohol use may also develop into abuse and dependence, respectively (APA, Diagnostic and Statistical Manual-IV). The societal ills and costs related to acute and long-term alcohol consumption have thus engendered a focused search for effective treatments (Altman, et al. 1996).

Drug use in humans is likely to be a multiply determined behavior incorporating the influence of psycho-social, biological, and economic factors (Altman et al. 1996; Cloninger, 1987). Interestingly, animals who are presumably less influenced by social and economic factors, essentially self-administer the same drugs as humans (DiChiara and Imperato, 1988; Schuster and Thompson, 1969). In general, the fact that animals and humans avoid and seek approximately the same type of drugs has strongly oriented research towards determining the biological basis of abused drugs, including alcohol by using animal models of the human behavior. A promising avenue of research has been the attempt to identify and understand the neurobiological factors which contribute to the initiation and maintenance of alcohol consumption (Amit and Smith, 1992; Koob, 1992).

The purpose of this introduction is to provide a brief review of various methods that have been critical in the search for the neural substrate mediating the reinforcing properties of abused drugs, such as ethanol. In addition, particular attention will be given to the dopamine neurotransmitter because of its general involvement in motivation (Jaber, Robinson, Missale, et al. 1996) and

drug self-administration (Wise, 1982). The terms reinforcement and reward will be used interchangeably to denote the effect of manipulations which increase the repetition of the behavior being performed (Carlson, 1986).

Several hypotheses and mini-theories have been formulated to explain the voluntary intake and abuse of drugs (e.g. Koob, 1992; Wise and Bozarth, 1987). A common approach employed in the early 20th century were investigations to identify the common neurobiological processes underlying the abuse of drugs such as cocaine, amphetamine and alcohol (Altman et al. 1996). Of the many theories proposed over the years, the hypothesis that the positive reinforcing properties associated with drug consumption strengthen and maintain the behavior, has received the most empirical support (Amit and Smith, 1992; Koob, 1992). Neurobiological processes, such as neurotransmitter release which occur when a drug is consumed are believed by some investigators to mediate its positive reinforcing properties (Wise and Bozarth, 1987). Thus, eliminating or reducing the action of a neurotransmitter by either the destruction of its pathway or by blocking its transmission should produce evidence about its role in regulating drug consumption (Amit and Smith, 1992).

Catecholamines and reward: anatomy and intracranial self-stimulation

Presently of historical value but still central to the issue of investigating the biological substrates underlying reward processes were an early series of animal studies undertaken by Olds and Milner (1954, 1956). These studies demonstrated that intracranial self-stimulation (ICSS) or electrical brain stimulation could function as a positive reinforcer. An animal's willingness to repeat a behavior such as a lever press in order to receive electrical brain stimulation and the fact that electrode placement in some brain regions and not

others were rewarding, argued in favor of the existence of specialized brain reward systems (Wise, 1978). The results of these studies indicated that electrical brain stimulation was as effective a reinforcer as many natural reinforcers such as food, sex and water (Koob, 1992). Further, the demonstration that electrical stimulation of hypothesized brain reward sites elicits behaviors such as eating and drinking implied that the neural substrates mediating rewarding brain stimulation and natural consummatory behaviors may be identical (Coons, Levak & Miller, 1965; Hoebel & Teitelbaum, 1962; Margules & Olds, 1962).

Three major types of evidence; studies on electrical brain stimulation (Olds & Milner, 1954), lesion studies (Epstein & Teitelbaum, 1967) and pharmacological studies (Brown, Gill, Abitbol & Amit, 1982) have implicated the catecholamines (CA) in reward mechanisms. Dopamine (DA), norepinephrine (NE) and epinephrine, three catecholamine neurotransmitters (Fuller, 1983) were postulated to be possible candidates for the biological mechanism underlying specialized brain reward systems (Poschel and Ninetman, 1963; Stein, 1962; Wise 1978). In addition, the use of improved neuro-anatomical techniques (Lindvall and Bjorklund, 1974; Ungerstedt, 1971) enabled the identification of major CA pathways in brain. Specifically, the medial forebrain bundle (MFB) with its overlapping projections of the dorsal and ventral noradrenergic (NE) bundles and the mesolimbic and nigro-striatal dopamine (DA) systems were hypothesized to be part of the pathways implicated in the reinforcement process (Lindvall and Bjorklund, 1974; Ungerstedt, 1971).

The NE system was initially hypothesized to be responsible for the rewarding effects of intracranial self-stimulation (Poschel & Ninteman, 1963; Stein, 1962). Intracranial self-stimulation (ICSS) was reported to occur along the trajectory of the dorsal tegmental NE bundle (DNB), whose fibers originate in the locus coeruleus (LC) (Crow, Spear, & Arbuthnott, 1972; Ritter and Stein, 1973).

However, the anatomical findings implicating NE in ICSS were later disputed because it was found that electrode placement in the LC nucleus proper, was not effective in eliciting changes in the rate of ICSS (Amaral and Routtenberg, 1975; Simon, LeMoal & Cardo, 1975). Theoretically, if NE was implicated in mediating ICSS reward then electrical stimulation of the anatomical structure containing the originating NE fibers should result in rewarding brain stimulation (Wise, 1978). A viable explanation for the failure of Amaral & Routtenberg and Simon's group to obtain LC stimulation was offered by Segal and Bloom (1976), who demonstrated that stimulation of the anterior pole of the LC nucleus was necessary to activate the DNB fibers and rewarding LC stimulation. Both groups, Amaral & Routtenberg, (1975) as well as Simon et al. (1975) had electrode placements in the posterior pole of the LC nucleus that activated NE fibers to the cerebellum (Segal and Bloom, 1974).

Lesion studies

Conceptually, destruction of catecholamine fibers should produce extinction of the behavior it once maintained (Carlson, 1986). Electrolytic lesions to DNB fibers produced low self-stimulation rates only for a day after the lesions (Corbett, Skelton & Wise, 1977). Neurotoxic lesions using 6-hydroxidopamine (6-OHDA), a specific-catecholamine neurotoxin to DNB fibers failed to disrupt LC stimulation despite 97% total cortical NE depletion (Clavier, Fibiger & Phillips, 1976). In addition, DNB self-stimulation was not disrupted by lesions to the LC (Koob, Fray & Iverson, 1978). These findings seriously questioned the notion that activation of NE fibers was critical to LC self-stimulation (Wise, 1978).

Lack of congruent support for NE's involvement in brain stimulation reward led to studying the effects of DA, a precursor to NE (Fuller, 1983). Dopaminergic involvement in brain reward was supported by anatomical

studies demonstrating rewarding brain stimulation at or near sites containing DA cell bodies, fibers and terminals (Crow, 1972; Phillips, Carter and Fibiger, 1976; Stein, 1969). The ventral tegmental area (VTA), and its DA projections to various sites such as the limbic system, nucleus accumbens, amygdala and frontal cortex, have frequently been sites supporting positive self-stimulation (Crow, 1972; Huang & Routtenberg, 1971; Phillips, Brooks & Fibiger, 1975; Routtenberg & Sloan, 1972). On the other hand, there have been studies which do not support the primary involvement of a dopaminergic mechanism in self-stimulation. For example, unilateral 6-OHDA injections into the nigro-striatal bundle, causing 97% depletion of DA, produced only transitory decreases in self-stimulation (Clavier and Fibiger, 1977). The authors thus concluded, it was unlikely that DA was involved in mediating brain reward since lesions to DA fibers only resulted in temporary effects on self-stimulation rates.

Early neuro-anatomical studies implicated the catecholamines in reward and helped delineate the preliminary anatomical boundaries believed to be involved in reinforcement processes (e.g.: Wise, 1978). However, the correlational nature of these studies was not sufficient to convince researchers that CAs were "the" neurochemical substrate of reinforcement (Olds, 1975). Anatomical studies suggested that CA neurotransmission was related to reinforcement but failed to establish a strong relationship between them. Focal brain lesions of CA structures failed to consistently produce disruptions in self-stimulation rates (Wise, 1978). As such, the correlation between anatomical structures containing CA and positive self-stimulation sites was weak.

The evidence for CA mediation of reward emanating from self-stimulation studies was not convincing (Olds, 1975). Several critical questions concerning the catecholamines' role in reward remained unanswered or even contradictory to the CA hypothesis of reinforcement. Specifically, if CAs were critical to the

mediation of reinforcement how could responding for self-stimulation be maintained in animals which have most of their cortical CA depleted? (Wise and Rompré, 1989). In other words, if the functional integrity of the CA system is necessary to mediate and sustain self-stimulation, then what could explain self-stimulation in animals no longer possessing functional CA neurotransmission?

Pharmacological studies and brain self-stimulation

The data reported on by studies of pharmacological manipulations of DA transmission on brain self-stimulation rates, may have provided the experimental evidence needed to support a claim for catecholaminergic involvement in brain reward, but controversy remained (Edmond and Gallistel, 1977). In general, the consensus was that self-stimulation was disrupted by drugs which inhibited CA function and facilitated by drugs which enhanced CA function (Edmond and Gallistel, 1977; Wise, 1978). A problem inherent in early self-stimulation studies was determining whether changes in rates of self-stimulation resulting from pharmacological manipulations of DA were due to changes in reinforcement and/or motor impairment (Valenstein, 1964). The difficulty in isolating and interpreting changes in self-stimulation rates was largely due to the fact that catecholamines also mediate a variety of behavioral functions, such as locomotor activity, emotion and affect (Jaber et al. 1996). Thus, drugs which impair CA function also have the capacity to produce decreased rates of responding that may be the result of the sedative effects of the drug (Rolls, Kelly & Shaw, 1974) or due to impaired motor function (Fibiger, Carter & Phillips, 1976).

Self-administered drugs: cocaine, amphetamine and the opiates

Intravenous (i.v.) self-administration of drugs in animals has frequently been employed as an alternate paradigm to ICSS, as a method to study the

behavioral effects of CA manipulations and to elucidate reinforcement processes (Bozarth, 1989). In this paradigm, a fixed-reinforcement schedule is typically used to train animals to lever press in order to obtain a drug injection delivered through an indwelling i.v. catheter. Decrements or increases in lever pressing for access to a drug are believed to parallel an attenuation or facilitation of a drug's reinforcing properties (Bozarth, 1989).

Prototypical agents readily self-administered by humans (DiChiara & Imperato, 1988; Schuster & Thompson, 1969) and animals (Weeks & Collins, 1987) are the psychomotor stimulants (e.g.: cocaine and amphetamine) and opiates (e.g.: heroin and morphine) (Bozarth, 1989). The psychomotor stimulants (Heikkila, Orlansky & Cohen 1975) and opiates (Axelrod, 1970; Carlsson, 1970) are both purported to enhance CA neurotransmission. Despite this fact, behavioral studies have focused on DA transmission as the critical element involved in mediating the reinforcing effects of stimulant self-administration (Wise and Bozarth, 1987). In general, support for DA's primary role in reinforcing stimulant use was based on studies demonstrating that drugs which blocked post-synaptic DA receptors decreased responding for cocaine (Woolverton, 1986) and/or produced an extinction-like pattern of responding (deWit and Wise, 1977), while drugs that blocked NE receptors failed to disrupt i.v. self-administration of amphetamine (Yokel and Wise, 1975).

The meso-limbic dopamine system, particularly the nucleus accumbens and the VTA, are sites that have been implicated in the mediation of the reinforcing effects of the psychomotor stimulants (Bozarth, 1989). Neurotoxic lesions of the nucleus accumbens (Lyness, Friedle & Moore, 1979; Roberts, Corcoran & Fibiger, 1977) and the VTA (Roberts and Koob, 1982) have produced an attenuation or cessation of stimulant self-administration of the indirect dopamine agonists amphetamine and cocaine.

As a psychoactive substance, the opiates have also been postulated to increase synaptic DA levels, albeit through a more complex system (e.g.: DiChiara, 1995). Systemic injections of opiates have been shown to increase activity in the dopamine cells of the VTA suggesting that DA may be involved in opiate reinforcement (Brodie and Dunwiddie, 1990). However, DA-mediated reinforcement of opiate administration has been a point of contention in the literature (Amit & Brown, 1982; Wise and Bozarth, 1982). The difficulty of implicating DA in opiate self-administration with the use of lesions (Dworkin, Guerin, Co, Goeders & Smith, 1988; Pettit, Ettenberg, Bloom & Koob, 1984) and DA blockers (Ettenberg, Pettit, Bloom & Koob, 1982; Gerrits, Ramsey, Wolterink & van Ree, 1994), has led several researchers to conclude that there appears to be little evidence supporting the notion that DA transmission is essential for reinforcing opiate self-administration (Amit and Brown, 1982; DiChiara, 1995).

Re-evaluating the DA hypothesis

Two diverging yet related lines of research within the field of neurobiology; molecular genetics and electrophysiology, have recently produced a flood of data which may eventually lead to a new conceptualization of DA's role in reward. In its simplest form, the DA hypothesis of reward assumed that once activated, the broader function of dopamine was to signal reward (Wise and Bozarth, 1987). However, this interpretation of dopamine's role in reward was questioned on the basis of recent voltametric and microdialysis studies. Phillips, Atkinson, Blackburn & Blaha (1993) employing the technique of microdialysis, measured the levels of brain DA in response to food rewards (Fiorino, Coury & Phillips, 1997) as well as to stimuli which predicted those rewards. These researchers observed that after animals learned to associate a stimulus to a

reward, the stimulus produced a similar increase in dopamine as the reward itself. Furthermore, the direct recording of DA cells in the substantia nigra (Schultz, Dayan & Montague, 1997) and the ventral striatum (Bowman, Aigner & Richmond, 1996) indicated that the DA cells fired in response to stimuli predicting reward and also in anticipation of a correctly executed task that will be rewarded. It appeared that dopamine neurons, as indicated by their brain concentrations (Phillips et al. 1993; Fiorino et al. 1997) and their cellular activity (Schultz et al. 1997) generally responded to "behaviorally significant stimuli" that correctly predicted reward. Notwithstanding the fact that there are flaws and disadvantages to the newer techniques employed to monitor DA transmission (DiChiara, 1995), this method has expanded our conception of DA's possible role in drug administration. Thus, dopamine's role in reward may be the subtler version of teaching an animal which responses are more effective in attaining reward, as opposed to relaying the message, that this substance is rewarding.

As a more in depth test of DA's involvement in modulating the reinforcement of abused drugs, researchers have developed genetically altered strains of mice lacking the dopamine transporter (DAT) gene, essentially eliminating the re-uptake mechanism for released dopamine (Rocha, Fumagalli, Gainetdinov, et al. 1998). The lack of the DAT mechanism translated into increased extracellular levels of DA as compared to control mice. Results obtained in recent studies employing DAT knockout mice, further questioned the central role attributed to dopamine, specifically in regulating the use of psychostimulants such as cocaine (Rocha et al. 1998). It was expected that without the DAT gene, animals would not acquire cocaine self-administration because cocaine would no longer have the capacity to increase DA levels thus eliminating the biochemical substrate for its reinforcing effect. Results indicated that despite the lack of DAT, the knockout mice preferentially self-administered

cocaine to saline. Further, the increase in cocaine self-administration was not attributable to increased responding due to psychomotor agitation (Rocha et al. 1998). These results are difficult to reconcile with the notion that psychostimulant reinforcement is mediated through increased DA transmission, as cocaine reinforced administration in DAT mice could not be attributed to a further increase of DA in extracellular space (Rocha et al. 1998).

The findings with the DAT mice in addition to cocaine's ability to increase 5-HT release through an inhibitory re-uptake mechanism (Reith et al. 1986) has led to a renewed interest in the involvement of a 5-HT reinforcement mechanism in drug administration (LeMarquand, Phil & Benkelfat, 1994; Rocha, Ator, Emmet-Oglesby & Hen, 1997). New developments in research implicating 5-HT in psychostimulant reinforcement are beyond the scope of this thesis and will not be discussed further. However, these studies do indicate an important area for future research. As recent developments have suggested, it will be important to focus on more than one neurotransmitter as the common final pathway because drug reinforcement is likely mediated by a complex multi-transmitter system (Amit and Smith, 1992; Koob, Roberts, Schulteis, et al. 1998).

Ethanol and reward

Neurochemical, electrophysiological and behavioral evidence have generally supported the hypothesis that dopamine, although not the primary link, may contribute to psychostimulant reinforcement (Koob, 1992). It was initially suggested that the behavioral and reinforcing effects of different classes of abused drugs, including alcohol, may be mediated by the activation of a common neural mechanism, likely dopaminergic in nature (Samson and Harris, 1992; Wise and Bozarth, 1987). Yet, attempts to specify the neurochemical and

neurophysiological nature of ethanol's reinforcement mechanism more precisely have not produced any conclusive evidence (e.g.: Smith and Amit, 1988).

The CAs have been postulated to subserve most motivated behaviors (German and Bowden, 1974; Stein, 1969) including drug consumption (Fibiger, 1978; Wise, 1978). In particular, the notion of CAs involvement in ethanol consumption was advanced by the finding that electrical stimulation of the lateral hypothalamus, an area traversed by major CA pathways (Ungerstedt, 1971), increased ethanol intake and preference for several months following termination of the stimulation (Amit and Stern, 1971; Amir and Stern, 1972; Atrens, Mairfaing-Jallat & Le Magnen, 1983). It was thus suggested that voluntary consumption of ethanol may be mediated by the catecholamines; dopamine and norepinephrine (Smith and Amit, 1988). A role for central CAs in ethanol consumption was further supported by the finding that administration of alpha-methyltyrosine (AMT), a CA synthesis inhibitor, produced transient reductions in ethanol intake (Myers and Veale, 1968). AMT has also been shown to prevent the re-acquisition of intragastric self-administration of ethanol in rats (Davis, Smith & Werner, 1978), as well as the euphoria induced by alcohol in humans (Ahlenius, Carlsson, Engel, Svensson & Sodersten, 1973).

Lesions have also been employed to destroy various brain structures thought to be involved in ethanol reinforcement in order to examine their effects on ethanol intake and to elucidate the substrates underlying ethanol reinforcement (Smith and Amit, 1988). Several studies have indicated that electrolytic lesions to the lateral hypothalamus (Amit, Meade, Levitan & Singer, 1976) as well as intraventrical lesions produced by the neurotoxin 6-hydroxydopamine (6-OHDA) (Brown and Amit, 1977; Myers and Melchior, 1975) reduced ethanol intake. Although, lesion studies implicated the CAs in mediating ethanol reinforced behavior it became apparent that a drawback in

these studies was the failure to delineate the relative importance of NE and DA (Smith and Amit, 1988).

It has been proposed that the integrity of the NE rather than the DA system was required to sustain ethanol reinforced behavior. Brown and Amit (1977) demonstrated that infusions of 6-OHDA intraventricularly depleted both DA (80%) and NE (87%) and significantly reduced ethanol preference. However, when NE neurons were protected by pre-treatment with desmethylimipramine (DMI), a NE-reuptake blocker, the same 6-OHDA lesions no longer disrupted patterns of ethanol intake. The differential effects of 6-OHDA lesions on ethanol self-administration were taken to imply a role for NE in reinforcing ethanol intake (Brown and Amit, 1977). More recent work (Corcoran, Lewis & Fibiger, 1983; Rassnick, Stinus & Koob, 1993) has shown that 6-OHDA lesions of mesolimbic DA pathways failed to disrupt oral ethanol intake as well as ethanol reinforced lever-pressing. The above results thus supported the notion that central NE systems, rather than DA systems, were involved in mediating ethanol reinforcement. However, a clear role for NE as opposed to DA-mediated ethanol reinforcement has been difficult to establish due to inconsistent findings obtained with the use of neurotoxins. For example, 6-OHDA lesions of NE pathways by Kiianmaa et al. (1975; 1980) not only failed to reduce ethanol intake as previously observed (e.g.: Brown and Amit, 1977; Corcoran et al., 1983), they produced transient increases. Furthermore, lesions produced with the specific NE neurotoxins 6-hydroxydopa (Richardson and Novakowski, 1978) and DSP-4 (Gill, Amit & Ogren, 1984) failed to alter ethanol intake. The failure to demonstrate reductions in voluntary ethanol intake when using specific NE neurotoxins did not preclude NE involvement in ethanol consumption (Gill et al. 1984). The results of these studies have been interpreted, in conjunction with the work of Zigmond and colleagues (1973; 1980; 1981), to suggest that destruction of

the NE pathway does not appear to functionally impair the entire NE system thus allowing the remaining NE system to compensate for lost functioning (Gill et al. 1984).

Studies using enzyme inhibitors to deplete brain NE have more consistently implicated central NE systems in mediating ethanol reinforced behavior. Depleting NE synthesis in brain with dopamine-beta hydroxylase (DBH) inhibitors has produced reductions in ethanol intake (Amit, Brown, Levitan & Ogren, 1977; Brown, Amit, Levitan, Ogren & Sutherland, 1977). DBH is an enzyme necessary for the synthesis of NE from DA (Amit et al. 1977). DBH inhibitors have also been shown to reduce lever pressing for intragastric infusions of ethanol (Davis, Smith & Werner, 1978; Davis, Werner & Smith, 1979).

It has been suggested that the discrepancy between the results obtained with lesions and enzyme inhibitors was linked to the fact that enzyme inhibitors produced longer lasting impairment to the NE system and thus recovery and compensation were unlikely to occur within the time frame of the experiment (Gill et al. 1984).

Neurochemical and neurophysiological monitoring of DA and ethanol

The position that the pharmacological effect of drugs of abuse arise mainly from their interaction with the mesolimbic DA system, particularly the VTA and the nucleus accumbens (Samson and Harris, 1992; Wise and Bozarth, 1987), has been the focus of much research and debate (Altman et al. 1996; Amit et al. 1982; DiChiara, 1995). It thus became important for researchers to actually demonstrate that ethanol's action(s) were primarily mediated by DA neurons in the VTA and nucleus accumbens. In recent years, the use of new methods to monitor and manipulate transmitter activity has enabled researchers to actually measure neurotransmitter levels in relation to ethanol intake. However, most

studies have tended to focus on measuring levels of DA while failing to investigate ethanol's actions on other neurotransmitters (Altman et al. 1996). Measuring the level of other neurotransmitters is vital to understanding ethanol's biochemical mechanism as ethanol is known to interact with several neurotransmitters within the central nervous system (CNS) including GABA, serotonin, catecholamines and the opiates (Bono, Balducci, Richelmi, et al. 1996).

In vitro (Brodie, Shefner & Dunwiddie, 1990) and in vivo (Gessa, Muntoni, Collu, Vargiu & Mereu, 1985) electrophysiological studies have reported that ethanol dose-dependently stimulated firing of dopamine containing cells in the VTA area (A10). Microdialysis studies have also suggested that systemic (Imperato and DiChiara, 1986) as well as locally perfused ethanol (Wozniak, Pert, Mele & Linnoila, 1991; Yoshimoto, McBride, Lumeng & Li, 1992) increased extracellular dopamine release in the nucleus accumbens. However, the question of whether increased DA activity was a necessary and sufficient condition to produce a reinforcement effect influencing ethanol consumption has not been resolved in the literature (e.g.: DiChiara, 1995).

It is important to remember that DA transmission is intimately linked to several behavioral functions, such as locomotor activity, emotion and affect (Jaber et al. 1996). For example, it is well established that ethanol has dose-dependent effects on locomotor activity (Durcan and Lister, 1988) Thus, ethanol at low doses was shown to stimulate motor activity in mice (Koechling, Smith & Amit, 1990) as well as in alcohol-preferring rats (Waller, Murphy, McBride, et al. 1986). Ethanol-induced motor excitation has been postulated to be linked to the increased activity of the CAs (Liljequist and Carlsson, 1978; Dudek, Abbott, Garg & Phillips, 1984; Menon, Dinovo & Haddox, 1987). The motor excitation induced by low doses of ethanol has been effectively blocked by a DA antagonist,

(Koechling, Smith & Amit, 1990; Shen, Crabbe & Phillips, 1995) and a NE antagonist (Koechling et al. 1990) suggesting that both DA and NE may be responsible for ethanol-induced motor excitation. In fact, the ability of NE blockers to suppress motor excitation after ethanol administration argues against a solitary role for dopamine in mediating ethanol-induced motor excitation (Koechling et al. 1990). On the other hand, it has been suggested that the motor excitation observed after ingestion of ethanol is an indication of the DA-mediated psychostimulant properties of abused drugs (Wise and Bozarth, 1987).

The ability of DA antagonists to suppress ethanol-induced motor excitation in mice has been interpreted to support, in part, a psychomotor stimulant theory of drug dependence (Wise and Bozarth, 1987). However, accepting this notion would lead one to accept the arguable premise that DA-mediated stimulation and ethanol's reinforcing effects are one and the same (Wise and Bozarth, 1987). Conceptually, support for this premise should only be extended when researchers can demonstrate that damaging and/or blocking dopamine transmission reliably disrupts voluntary ethanol consumption. Without supporting behavioral evidence from self-administration studies the value of the physiological evidence is weakened because DA release (Imperato and DiChiara, 1986) and cellular activity (Brodie, Shefner & Dunwiddie, 1990) may be a secondary effect to, and not a direct effect of, ethanol reinforcement (DiChiara, 1995).

Paradigms of ethanol self-administration

Ethanol's capacity to reinforce behavior is generally accepted (Amit and Stern, 1971; Samson, Pfeffer & Tolliver, 1988) and was demonstrated in animals that learned to perform an operant in order to gain access to ethanol solutions (Amit and Stern, 1971). Ethanol was initially believed to be a weak reinforcer

(Winger, Young & Woods, 1983) when examined using i.v. administration procedures. However, the I.V. route of administration was criticized as "an inappropriate model of human use" and thus the development of oral self-administration procedures became necessary (Amit, Smith & Sutherland, 1987; Samson, Pfeffer & Tolliver, 1988).

The use of oral self-administration paradigms to evaluate the reinforcing properties of ethanol has not been without controversy (Cicero, 1980; Deitrich and Melchior, 1985; Lester and Freed, 1973). Oral paradigms have been criticized as inappropriate models of alcoholism because they frequently fail to induce ethanol drinking to the point of intoxication and generally do not create signs of dependence following withdrawal of ethanol (Amit et al. 1987). In an attempt to overcome these criticisms several methods to induce animals to drink larger amounts of ethanol have been developed. The most commonly employed methods to induce ethanol consumption include an alternate day schedule of ethanol presentation (Amit, Stern & Wise, 1970), a sucrose-fading procedure (Samson and Pfeffer, 1970), as well as gradually increasing ethanol concentrations (Myers and Veale, 1972). Criticisms of the use of oral self-administration paradigms in animals overlooked two important facts. First, the primary route of ethanol administration in humans is oral, thus it makes sense conceptually to attempt to employ similar routes of administration in animals. Second, other routes of administration (e.g.: i.v.) which have been used to investigate the abuse liability of other drugs such as cocaine have generally failed to produce elevated and consistent levels of ethanol administration (Gill, 1989; Numan, 1981).

Continuous and limited access paradigms

When oral-self administration paradigms are used to investigate the biochemical substrate underlying ethanol intake, dopamine's role in mediating ethanol reinforcement does not appear convincing (Amit and Brown, 1982). In general, results supporting the involvement of DA in the reinforcement of voluntary ethanol consumption have been equivocal (e.g.: Levy and Ellison, 1985; Mudar, LeCann, Czirr et al. 1986; Pfeffer and Samson, 1988). Furthermore, the methodology employed in conjunction with oral self-administration paradigms has varied tremendously and thus renders comparisons across studies, difficult at best. The failure of researchers to regulate the methodology employed in self-administration studies has meant that studies often vary by rat strain, acquisition paradigm, ethanol's exposure time, response requirements for access and ethanol concentration (Gill, 1989). Thus methodological differences across studies have likely contributed to the divergent results obtained. As the self-administration literature is vast and varied, the focus of this thesis will be on oral self-administration studies employing various access paradigms which examined the effect of dopaminergic agents on ethanol consumption.

Psychopharmacological studies manipulating DA function to examine its effect on voluntary ethanol consumption have employed a variety of dopaminergic agents, specific to the D2 receptor subtype (Brown et al. 1982; Linesman, 1990; Pfeffer & Samson, 1986). In early studies, it was suggested that the D2 receptor site specifically was linked to the mediation of reinforcement (deWit and Wise, 1977). For example, antagonists with primary affinity to the D2 receptor, such as pimozide have been shown to block lever pressing reinforced with cocaine (deWit and Wise, 1977). However, pharmacological manipulations of the DA system to examine the effects on oral self-administration of ethanol have at best provided evidence which appeared to be paradigm specific

(Linesman, 1990). In other words, positive effects have typically been obtained more consistently within one paradigm as compared with the other (Pfeffer and Samson, 1986; Brown et al. 1982).

Continuous access, D2 agents and ethanol consumption:

Two types of procedures used to examine the effects of pharmacological manipulations on ethanol consumption are continuous access and limited access. The amount of time an animal has access to an ethanol solution distinguishes continuous and limited access. Continuous access typically provides 22-hr to 24-hr of unrestricted access (e.g.: Brown, et al. 1982) to ethanol while limited access provides short access periods varying from 30 minutes up to 4-hrs, depending on the study (e.g.: Linesman, 1990; Dyr, McBride, Lumeng, et al., 1993). The choice of paradigm has often been dictated by the time course of a drug's action. It has been suggested (Linesman, 1990; Files, Lewis & Samson, 1994) that the effects of short-acting drugs are better examined within a limited access paradigm when the peak drug effect coincides with ethanol presentation.

A continuous access paradigm was employed by Brown et al. (1982) where rats were provided with 24-hr unrestricted access to ethanol and water in a two-bottle choice procedure. Fluid consumption was measured at the end of the access period. Administration of pimozide at the start of the 24-hr period failed to alter consumption of ethanol (g/kg) or water over a 5-day period. In a recent study, Goodwin, Koechling, Smith & Amit (1996) used both rats from Maudsley Reactive strain, bred for high ethanol consumption, as well as Wistar rats to further examine the effect of dopamine blockade on ethanol consumption. Dopamine receptor blockade with pimozide produced an overall reduction in fluid consumption and hence no evidence for any differences specific to ethanol intake and/or preference across rat strains. The only study which found that

pimozide reduced home cage ethanol drinking (Pfeffer and Samson, 1986) failed to examine its effect on water intake thus precluding a statement on the specificity of this effect. Taken together, these results argued against a specific role for dopamine in mediating the positive reinforcing effects of ethanol when examined within a continuous access paradigm. A potential limitation of the continuous access paradigm was the possibility that an effect occurred but was not accurately detected due to the length of time between drug administration and measurement of fluid consumption (Pfeffer and Samson, 1988).

Limited access, D2 agents and ethanol consumption

Those researchers who did observe effects of dopamine antagonists on ethanol reinforced behavior have done so exclusively within limited access paradigms. Pfeffer and Samson (1985, 1988) have shown that both pimozide and haloperidol reduced operant responding for ethanol only when access was limited to a 30-min session. These authors suggested that observing decrements in ethanol reinforcement depended on the length of the drinking session. However, it has been pointed out that these agents also have general effects on consummatory behavior and therefore raised questions about the specificity of the obtained reduction in ethanol intake (Linesman, 1990; Spivak and Amit, 1985). In addition, Boyle, Spivak and Amit (1994) suggested the possibility that the "amount of ethanol consumed in the limited access paradigm in part reflected a compensatory response to the restricted availability of ethanol". Thus, a greater amount of ethanol consumed during limited access due to its restricted availability could artificially produce an effect during treatment as any decrease would likely be more pronounced under these conditions.

Curiously, reductions in ethanol reinforced responses have also been reported with DA agonists such as SDZ-205,152 (Rassnick, Pulverenti & Koob,

1993) and amphetamine (Levy and Ellison, 1985) during limited access. It has been argued that DA agonists produce decreases in ethanol intake similar to the decreases observed in cocaine intake (Koob and Weiss, 1990), as a result of a blockade of DA-mediated ethanol reinforcement. Thus, animals stop responding for ethanol because the agonist is said to now substitute for ethanol's reinforcing effect (Samson , Hodge, Tolliver et al, 1993). It thus appeared that effects of dopaminergic agents on ethanol intake were only obtainable within a limited access paradigm. However, the accuracy of this premise was questioned by an important study conducted by Linesman (1990).

Linesman (1990), used a limited-access paradigm in which rats had unlimited access to water and food, but received a one-hour session where access to ethanol and water was provided in two Richter tubes. Several dopamine antagonists and agonists were administered to different groups 30 minutes prior to ethanol presentation. In this study, a reduction in ethanol intake was observed following the administration of the antagonist haloperidol. However, it was suggested that the effects were non-specific to ethanol as a similar decrease was found in water consumption. In addition, Mudar, LeCann, Czirr et al., (1986) have shown that pre-treatment with the antagonist pimozide failed to alter consumption of a sweetened ethanol solution during a 1-hr access period. Furthermore, administration of nomifensine, a DA re-uptake inhibitor, failed to alter ethanol intake (Daoust, Moore, Saligaut, Lhuintre, Chretien et al., 1986). These results suggested that DA may not be involved in the mediation of ethanol reinforced behavior, regardless of whether it was examined within a limited access paradigm or any other paradigm for that matter.

The controversy as to whether dopamine specifically regulates the oral self-administration of ethanol remains at present unsettled. The body of data

generated by investigators with the use of animal models has generally been consistent within the paradigm being tested but inconsistent across different paradigms. In general, findings have suggested that D2 antagonists have failed to attenuate ethanol consumption when examined within a continuous access paradigm (Brown et al., 1982; Goodwin et al., 1996; Pfeffer and Samson, 1986). Greater success has been achieved with the use of dopaminergic agents within a limited access paradigm (Pfeffer and Samson, 1985; 1988; Rassnick et al., 1992). However, this finding is far from consistent and the possibility of motor involvement and/or general effects on fluid consumption question the specific role which can be attributed to DA as it relates to voluntary ethanol consumption.

Overall, attempts to identify the dopamine D2 receptor as the biochemical site of action mediating the reinforcement of ethanol have produced equivocal results (Aalto and Kiianmaa, 1986; Brown et al., 1982; Daoust et al., 1986; Goodwin et al., 1996; Linesman, 1990; Mudar et al., 1986). The more recently discovered dopamine D3 receptor has been shown to be expressed in the limbic system (Sokoloff, Giros, Martes et al., 1990) and has been suggested to mediate drug seeking behavior (Schwartz, Diaz, Griffon, et al., 1994). The possibility exists that clear pharmacological effects of dopamine manipulations on ethanol reinforcement were not observed thus far due to the previous unavailability of drugs which bound preferentially to the D3 receptor. Pure D3 dopamine receptor antagonists remain to be clearly identified, although 7-OH-DPAT a proposed D3 receptor agonist, has initially been shown to reduce preference for ethanol (Meert and Clincke, 1994). These researchers thus suggested that the D3 receptor, alone or in combination, may also mediate ethanol reinforcement (Meert and Clincke, 1994).

Mixed D2/D3 receptors and ethanol intake

To date there have been only a few studies which have investigated the effects of mixed D2/D3 agents on ethanol consumption within an oral self-administration paradigm. Dyr et al., (1993) employed alcohol-preferring HAD rats to examine the effects of subcutaneous injections of the mixed D2/D3 agonist, quinpirole (0.04, 0.08, 0.024, 0.5, 1.0, 2.0 mg/kg) on ethanol drinking throughout a 4-hr limited access period. The administration of quinpirole resulted in some selective (0.08 - 0.25 mg/kg) reductions in ethanol consumption, while not significantly decreasing food or saccharin intake. These researchers concluded that activation of the dopamine receptors decreased ethanol intake by "substituting for the reinforcing stimulatory effects of ethanol in alcohol-preferring rats" (Dyr et al., 1993).

Silvestre, O'Neill, Fernandez and Palacios (1996) employed a 24-hr access paradigm to test the effect of several dopaminergic agents on ethanol consumption in a two-bottle choice procedure. The lowest dose of 7-OH-DPAT, which they classify as a mixed dopamine D2/D3 agonist, reduced ethanol intake without affecting water intake. However, all doses (0.01, 0.1, 1.0 mg/kg) of the D2/D3 antagonist raclopride failed to alter ethanol intake without producing a similar effect on water intake. These results were interpreted to suggest a role for the D2/D3 receptors in mediating ethanol intake and reinforcement. However, several methodological issues may confound this interpretation. Silvestre et al., (1996) used a 10% ethanol solution that was distilled with 3% sucrose, in food restricted animals. Furthermore, drugs were administered twice daily with one drug administration period occurring during the 1-hr food access. These methodological factors thus increase the likelihood of confounding motivational factors. More importantly, the 1.0 mg/kg dose of raclopride is high and has been

shown (Hillegaart and Ahlenius, 1987) to produce extrapyramidal effects, yet, Silvestre et al. (1996) state that motor suppression was not an issue in the study, as only doses higher than 1.0 mg/kg have been shown to cause sedation.

Data collected from studies employing microinjections of DA antagonists and DA agonists into the nucleus accumbens and VTA has been cited as further evidence supporting DA involvement in ethanol reinforcement. Rassnick, Pulverenti and Koob (1992) have shown that the dopamine D2/D3 antagonist fluphenazine administered systemically and with intra-nucleus accumbens injections decreased lever pressing for access to ethanol. These authors suggested that the DA neurons in the nucleus accumbens may be involved in mediating the reinforcing effects of ethanol.

In contrast with the previous finding, the D2/D3 agonist, quinpirole has been shown to have no effect on ethanol reinforced responding when infused into the nucleus accumbens (Hodge, Haraguchi, Erickson and Samson, 1993). As well, quinpirole administration has decreased ethanol and sucrose reinforced operant responding with infusions in the VTA (Hodge et al. 1993). In addition, it has been demonstrated that raclopride, a mixed D2/D3 dopamine antagonist, when injected into the nucleus accumbens produced dose-related decreases in total responding for ethanol (Samson, Hodge, Tolliver and Haraguchi, 1993) and sucrose (Hodge, Samson, Tolliver and Haraguchi, 1994). However, the direction of the effects obtained with DA agonists and antagonists are not consistent as differential effects have been obtained depending on the route of administration employed. Pfeffer and Samson (1986) observed decreases in ethanol reinforced responding with systemic administration of d-amphetamine while microinjections have increased ethanol reinforced responding (Samson et al. 1993).

The present investigation

The purpose of this thesis is thus to further examine the role of dopamine D2 and D3 receptors in mediating voluntary ethanol consumption across two types of access paradigms. The dopamine D2 and/or D3 receptor subtypes have been postulated to mediate the reinforcing properties of voluntary ethanol consumption (Meert and Clincke, 1994; Silvestre et al. 1996). Thus, quinpirole a mixed D2/D3 agonist and raclopride a mixed D2/D3 antagonist were employed to determine if the D2 and D3 dopamine receptor subtypes have a role in maintaining ethanol consumption in an unselected rat strain. At present, there are no known studies which have examined the effects of systemically administered mixed D2/D3 agents on voluntary ethanol intake within both a continuous and a limited access paradigm. Of particular interest, was the attempt to determine if changes in ethanol intake after dopaminergic manipulations were a function of the characteristics of the limited access paradigm, specifically. In addition, it was necessary to assess whether any decrease in ethanol intake was a direct effect of dopaminergic mediation of ethanol reinforcement or rather of motor depression. Therefore, the purpose of the present experiment was to assess the generalizability of the effects of dopamine D2 and D3 agents on ethanol intake by examining their effect within both a continuous and limited access paradigm.

Experiment 1

The purpose of this experiment was to investigate the possible role of dopamine in mediating voluntary ethanol consumption in rats by examining the effects of a mixed D2/D3 dopamine agonist as well as a mixed D2/D3 antagonist on ethanol intake within a 1-hr limited access paradigm. Activation of common neurochemical pathways, particularly the mesolimbic DA system has been linked to the reinforcing properties of abused drugs (Koob, 1992). It has also been suggested that ethanol consumption in rats may be maintained at least in part through a dopaminergic mechanism of reinforcement (Pfeffer and Samson, 1988; Samson and Harris, 1992; Wise and Bozarth, 1987). The mechanism of action was believed to be linked to the dopamine D2 (Koob, 1992) and the dopamine D3 receptors (Sokoloff et al. 1990) which have been implicated in the mediation of drug reinforcement (Schwartz et al. 1994) in general and are also believed by some investigators to be linked to ethanol reinforcement (Hodge et al. 1996; Meert and Clincke, 1994). On the other hand, several researchers have disputed DA's primary role in ethanol reinforcement and have instead proposed other options, for example that acetaldehyde, the primary metabolite of ethanol, may mediate the reinforcing properties of oral self-administration (Brown, Amit, & Smith, 1978; Myers & Veale, 1975).

As observed with prototypical drugs of abuse such as cocaine, ethanol has been shown to increase extracellular dopamine levels as measured by neurochemical and neurophysiological methods (Gessa et al. 1985; Imperato and DiChiara, 1986; Yoshimoto et al. 1991). Thus, according to a dopamine hypothesis of reinforcement, it was expected that facilitation and/or inhibition of DA transmission should produce observable effects on ethanol intake. Yet, studies examining the effects of dopaminergic manipulations on oral self-administration of ethanol have not produced consistent results. Systemic

injections of dopamine agonists has resulted in decreases (Dyr et al. 1993; Weiss et al. 1990) and/or no change (Aalto and Kiianmaa, 1986; Daoust et al. 1986) in ethanol intake, while microinjections of DA agonists in reward relevant brain structures, such as the nucleus accumbens, has produced increases (Hodge et al. 1993) or no effect (Samson et al. 1993) on ethanol intake. The effect of dopamine antagonists administered systemically has been to decrease (Panocka et al. 1993a; b) and/or not change ethanol intake (Brown et al. 1982; Goodwin et al. 1996; Linesman, 1990). Intra-accumbens microinjection of DA antagonists has decreased (Rassnick et al. 1992) or enhanced ethanol intake (Levy et al. 1991).

Examining the effects of dopaminergic agents on ethanol consumption within a limited access paradigm, typically produces more coherent results (Smith and Amit, 1992). It is likely that the effects of dopaminergic drugs on ethanol reinforced behavior are more potent and observable when examined shortly after drug administration. Despite this observation, clear effects of dopaminergic manipulations on reinforcement and thus on ethanol consumption within limited access are at times difficult to assess since general decreases in consummatory behavior have also been found (Linesman, 1990).

If dopaminergic activation is important in order for an organism to learn about ethanol's reinforcing effects, then potentiating dopamine transmission with quinpirole, a mixed D2/D3 agonist should increase ethanol intake relative to controls. Additionally, blocking dopamine receptors with raclopride, a mixed D2/D3 antagonist should decrease but not eliminate ethanol intake relative to controls. However, if the effect of facilitating or inhibiting dopaminergic activity primarily interferes with the motor capacity of animals then ethanol intake should be practically non-existent during the treatment phase.

METHOD

<u>Subjects</u>

Subjects were 30 male Long-Evans rats (Charles River, Quebec), weighing 225-250 grams when received. Animals were housed individually in hanging stainless steel cages with unrestricted access to food (Purina Rat Chow). Fluids were provided by two Richter tubes attached to the front of the cage. Water availability was unrestricted except during the ethanol drinking initiation phase and one hour limited access. The colony room was maintained and controlled for temperature, humidity and a 12/12 h light/dark cycle (lights on at 0800). All experimental sessions were carried out during 1400 and 1600 hours of the light portion of the cycle.

<u>Drugs</u>

The 0.5 mg/kg dose of raclopride (l-tartrate) and 0.1 mg/kg dose of quinpirole (Research Biochemicals Inc.) were dissolved in a vehicle of 0.9% saline. Drugs were injected in a volume of 1.0 ml/kg body weight. Injections were administered subcutaneously 30 minutes prior to ethanol access. The dose of the drugs used were in the ranges previously established in the literature as either effective (e.g.: Dyr et al., 1993; Sylvestre et al., 1996) and/or the dose at which motor suppression effects begin to emerge (Hillegaart and Ahlenius, 1987). Ethanol solutions were prepared from a 95% ethyl alcohol stock and diluted with tap water to the appropriate concentrations of 2, 4, 6, 8 and 10% (v/v).

<u>Training Procedure</u>

Free access to food and water was first provided for a 10-day period in which animals were handled daily to promote habituation to laboratory conditions. Animals were then exposed to a forced-choice ethanol intake paradigm designed to facilitate the acquisition of ethanol consumption. The sole

fluid presented for a period of four consecutive days was a 10% (v/v) ethanol solution. Following this period, animals received alternate-day free-choice of a 10% ethanol solution and water over 29 presentations. On intervening days water was the only fluid available. Side of fluid presentation was alternated throughout training and experimental phases to limit the influence of a position preference. Fluid consumption was measured to the closest ml daily and converted to g/kg over the final five presentations of free-choice in order to determine individual ethanol intake. The amount of absolute ethanol (g/kg) consumed was then employed to rank and assign animals to one of three drug groups (n=10). The three groups were randomly designated as a raclopride group, a quinpirole group and a saline (control) group. Prior to introducing a limited-access schedule, animals were maintained on food and water for sixteen days. Animals were subsequently given daily one-hour limited access to increasing concentrations of ethanol. Water was available at all other times. Starting with a 2% solution, ethanol concentrations were increased by 2%, following every second ethanol presentation up to the final concentration of 10%. A 10% solution was then presented for five consecutive days to determine stable ethanol intake.

Experimental Procedure

Once ethanol intake was stabilized in the limited access schedule, the four day baseline phase began. Baseline consisted of a daily saline injection administered subcutaneously (sc.) to all animals 30 minutes prior to ethanol access. Animals received daily one-hour access to ethanol. Water was not available during this time. The baseline condition attempted to control for a vehicle effect. The four days following baseline constituted the treatment phase of daily drug injections administered sc. 30 minutes prior to ethanol access. Injections of either 0.5 mg/kg raclopride (n=10), 0.1 mg/kg quinpirole (n=10) or

saline (n=10) were given according to the assigned group. Saline injections were given in a volume equivalent to drug injections. A four day post-treatment phase was included in which animals were given saline injections sc. 30 minutes prior to ethanol access to detect if a return to baseline occurred.

RESULTS

In the present experiment, changes in ethanol consumption relative to saline controls and across phase were examined. In order to determine potential group differences in ethanol consumption, separate 2 (drug group) x 3 (phase) analysis of variance (ANOVAs) with the last factor as a repeated measure, were computed for each drug. Simple main effects and simple comparisons were employed to determine the source of interactions. Tukey post hoc analyses were employed to investigate significant main effects. An alpha level of 0.05 was used for all statistical tests.

Quinpirole

Data for mean ethanol consumption (g/kg) during 1-hr access, across baseline, treatment and post-treatment periods for the quinpirole group are presented in Figure 1. The main effects of drug [$F_{(1, 18)} = 0.627$], and phase [$F_{(2, 36)} = 0.820$] were not significant indicating that quinpirole-treated rats did not differ in their level of ethanol intake as compared to the control group. A significant interaction between drug group and phase period [$F_{(2, 36)} = 4.013$, $p < 0.05$] was investigated with a test of simple main effects. The analysis did not reveal any simple effects of drug group or phase period indicating the interaction effect was due to inherent variability in ethanol intake (g/kg) among subjects.

Raclopride

The ANOVA revealed a significant main effect of drug treatment [$F_{(1, 18)} = 10.465$, $p < 0.01$)] reflecting the finding that raclopride-treated rats drank significantly less ethanol (g/kg) than the control group (see Figure 2). A significant effect of phase period [$F_{(2, 36)} = 54.612$, $p < 0.001$), and an interaction of drug group and phase period [$F_{(2, 36)} = 37.198$ $p < 0.001$) were obtained. The
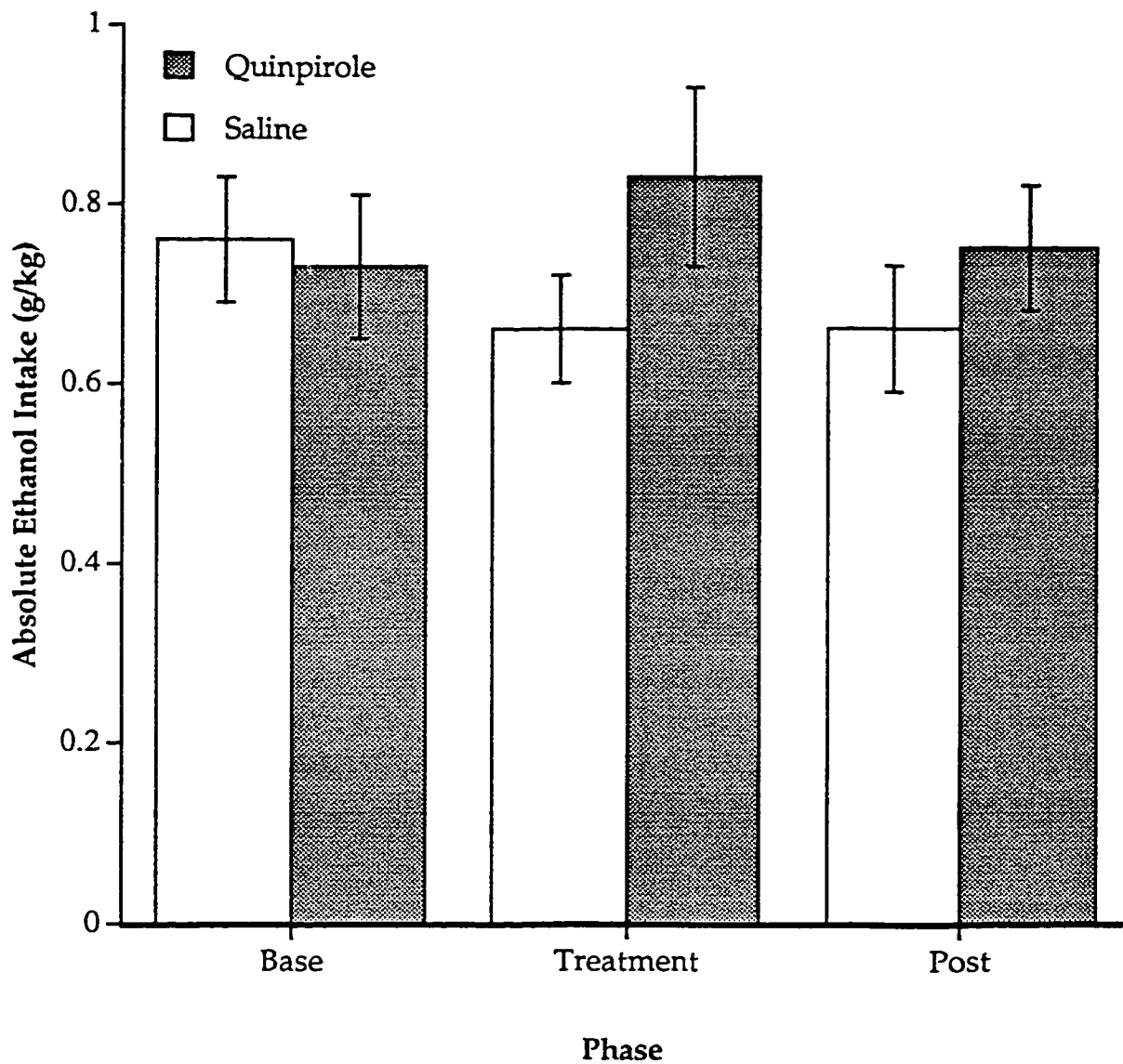
Figure 1. The effect of quinpirole on mean ethanol intake across 4-day phases. Absolute ethanol intake (g/kg) during a 1-hr limited access period is presented. Vertical bars represent S.E.M.
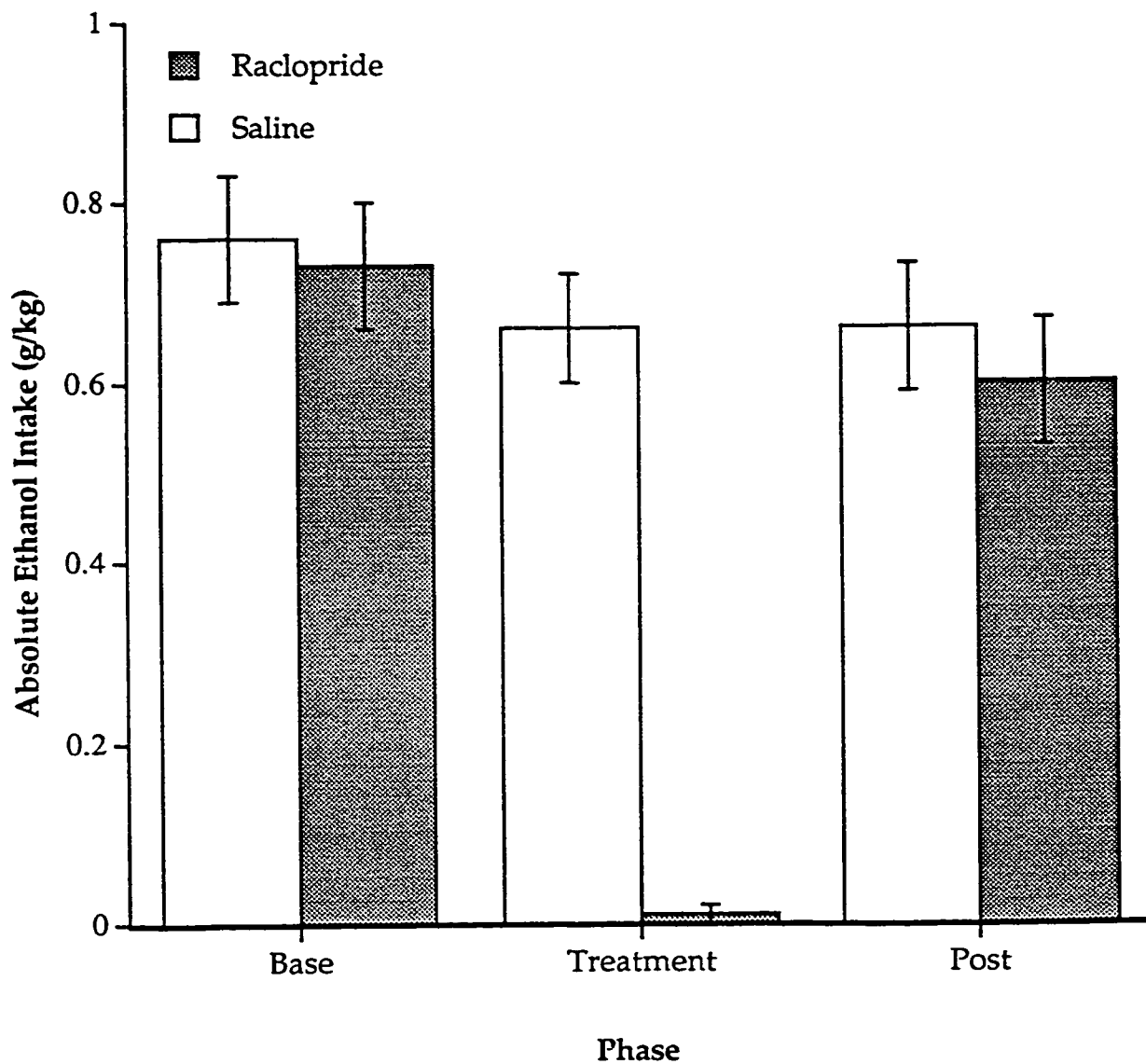
Figure 2. The effect of raclopride on mean ethanol intake across 4-day phases. Absolute ethanol intake (g/kg) during a 1-hr limited access period is presented. Vertical bars represent S.E.M.

analysis of simple effects reflected the finding that raclopride-treated rats drank significantly less ethanol than saline-controls across phases [(F $(2,36)$ = 89.806, p < 0.001)]. Analysis of simple comparisons revealed that ethanol intake during the drug phase was significantly reduced compared to baseline and post periods (p < 0.05).

DISCUSSION

The results of the first ANOVA revealed that the D2/D3 agonist quinpirole did not have a significant main effect on ethanol intake (g/kg) within a limited access paradigm. The results indicated an interaction of drug group and phase which influenced ethanol intake. However, further analysis of the interaction did not detect any significant effect within levels of the drug or phase factor. Thus, differences in ethanol intake across phase were probably the consequence of increased variability among subjects as opposed to a clear drug effect. The lack of effect of quinpirole on ethanol intake within the limited access paradigm observed in this experiment is inconsistent with previous findings (e.g., Dyr et al. 1993) which did support the involvement of dopamine in oral ethanol reinforcement.

The results of the second ANOVA revealed that the D2/D3 antagonist raclopride had a significant main effect on ethanol intake (g/kg) as compared to saline controls. Of particular interest, was the finding of decreased ethanol intake during the treatment phase observed in all raclopride-treated rats. Gross observation of animals for a 2-hr period post-drug treatment, indicated that raclopride-treated animals were ataxic and therefore did not consume ethanol.

Overall, this experiment showed that animals treated with quinpirole during the treatment phase failed to drink less than saline controls. The

raclopride-treated animals drank less ethanol (g/kg) during the treatment phase but observations indicated they were ataxic for the duration of the 1-hr limited access. Thus, the failure of quinpirole to increase ethanol intake combined with raclopride's non-specific effect on ethanol intake being linked to motor inhibition argues against the primary involvement of dopamine in oral ethanol mediated reinforcement. However, the possibility still remained that the decrease in ethanol intake in the raclopride group was nevertheless a result of dopamine-mediated reinforcement but that the effect could be observed only after the animals recovered from the motoric effects of the drug. Therefore, the next experiment was designed to address this possibility by examining raclopride's effect on ethanol intake within a continuous access paradigm. Similarly, it was important to determine if the effect of quinpirole on ethanol intake within a continuous access paradigm were similar to those obtained within a 1-hr limited access paradigm.

Experiment 2

Experiment 1 showed that the D2/D3 agonist quinpirole did not increase ethanol consumption while the D2/D3 antagonist raclopride attenuated ethanol consumption, when examined within a limited access paradigm. However, since the animals in the experiment were observed to be ataxic, it was important to determine whether the effects on ethanol were paradigm specific. In addition, it was necessary to assess whether the decrease in ethanol intake was an effect of dopaminergic mediation of ethanol reinforcement or of motor depression. If the effects of dopaminergic manipulations on ethanol intake were a function specifically, of the characteristics of the limited access paradigm, then it would be expected that a different intake pattern would be observed within another type of access paradigm. However, if the effects of dopaminergic manipulations on ethanol intake are not constrained to the type of paradigm employed, then it would be expected that results obtained within a continuous access paradigm would be similar to those obtained within limited access. Therefore, the purpose of the present experiment was to assess the generalizability of the effects of dopaminergic agents on ethanol intake by examining their effect within a continuous access paradigm. The same procedure was used as in Experiment 1, except that 22-hr continuous access to ethanol was provided instead of a 1-hr limited access.

Method

<u>Subjects</u>

Animals from Experiment 1 were the subjects in this experiment. There was a washout period of 31 days between the two experiments. Animals weighed 500-700g at the start of the experiment. Housing conditions were the same as in Experiment 1.

<u>Drugs</u>

Raclopride (0.5 mg/kg) and quinpirole (0.1 mg/kg) were prepared as in Experiment 1. A 10% (v/v) ethanol solution was the only concentration employed and it was prepared as in Experiment 1.

<u>Training Procedure</u>

Since animals were already established ethanol drinkers, a four day continuous free-choice schedule with 10% ethanol and water was employed. Animals were provided with continuous access to ethanol and water except for a two-hour period allotted for measurement. Side of fluid presentation was alternated throughout training and experimental phases to limit the influence of a position preference. Fluid consumption was measured to the closest ml daily to determine if consumption was stable.

<u>Experimental Procedure</u>

The procedure used was identical to that described in Experiment 1 except that access to ethanol was continuous over a 22-hr period.

RESULTS

In the present experiment, changes in ethanol and water consumption relative to saline controls and across phases were examined. In order to determine potential group differences in ethanol and water consumption, multiple 2 (drug group) x 3 (phase) analysis of variance (ANOVAs) with the last factor as a repeated measure, were computed for each drug. Simple main effects and simple comparisons were employed to determine the source of interactions. Tukey post hoc analyses were employed to investigate significant main effects. An alpha level of 0.05 was used for all statistical tests.

Quinpirole

Ethanol intake (g/kg) across phases is presented in Figure 3. The analysis of variance revealed no significant main effect of group [F (1, 18) = 0.417, p > 0.05], or phase [F (2, 36) = 1.123, p > 0.05], and no significant interaction [F (2, 36) = 2.737, p > 0.05]. These findings suggested that quinpirole treatment did not have an effect on ethanol intake, supporting the effect of quinpirole observed in Experiment 1.

Accordingly, there was no main effect of group [F (1, 18) = 1.253, p > 0.05], or of phase [F (2, 36) = 1.220, p > 0.05], and no significant interaction [F (2, 36) = 2.426, p > 0.05] as measured by the volume of ethanol(mls) ingested, again suggesting that quinpirole had no effect of ethanol intake. Similarly, water intake (mls) did not significantly differ between drug treatment groups [F (1, 18) = 0.325, p > 0.05], or phases [F (2, 36) = 2.705, p > 0.05]. There was also no significant interaction [F (2, 36) = 0.815, p > 0.05].

Total fluid intake (mls) was not significantly different between drug groups [F (1, 18) = 0.042, p > 0.05]. A main effect of phase [F (2, 36) = 11.034, p < 0.001] reflected differences in total fluid intake across phases (Figure 4). Post hoc analysis revealed that both groups had increased their total fluid intake during
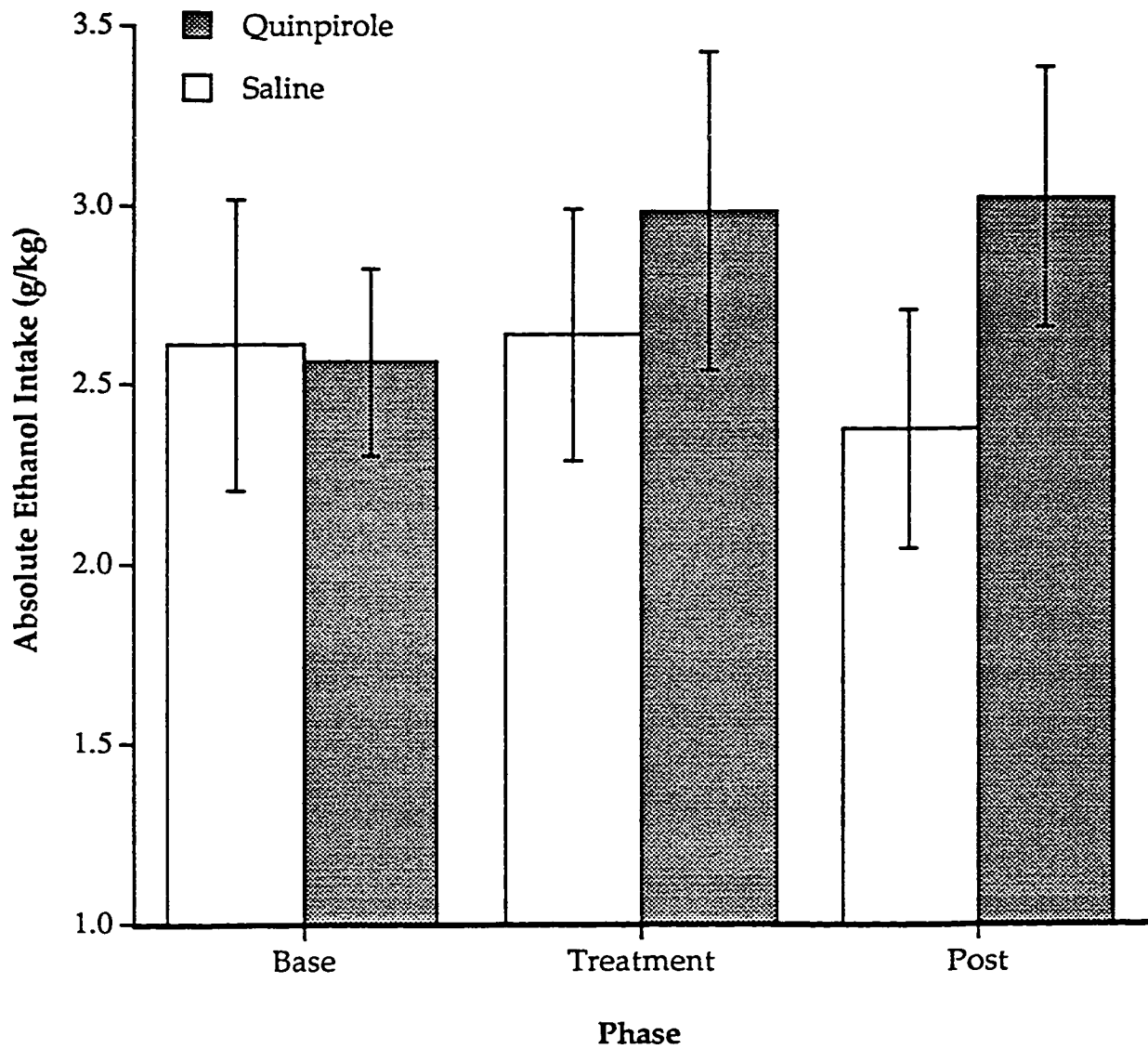
Figure 3. The effect of quinpirole on mean ethanol intake across 4-day phases. Absolute ethanol intake (g/kg) during continuous access is presented. Vertical bars represent S.E.M.
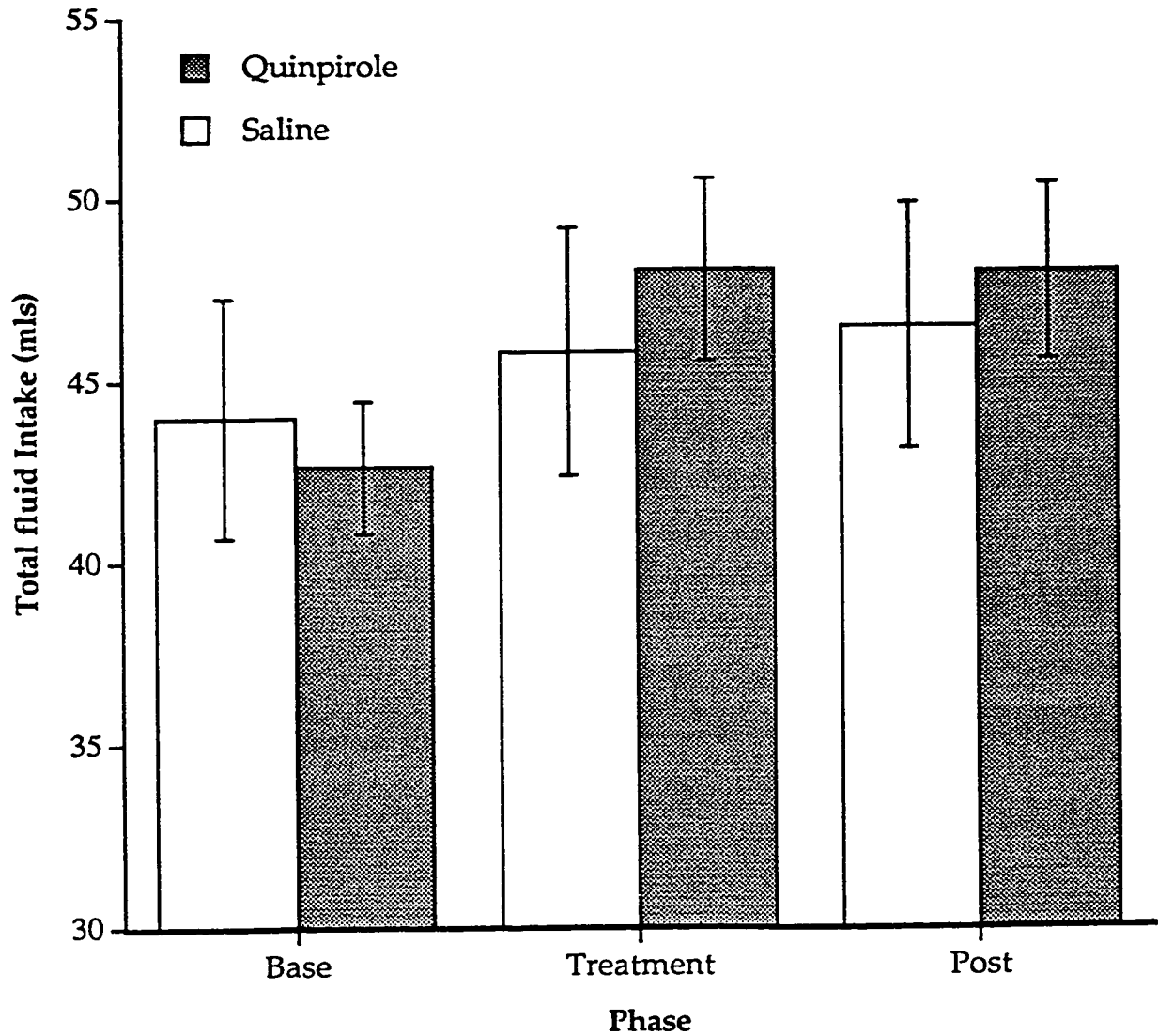
Figure 4.   Effect of quinpirole on total fluid intake across 4-day
            phases. Total fluid intake (mls) represented. Vertical lines
            represent S.E.M.

treatment and post compared to baseline (p < 0.05) indicating a general fluctuation in fluid intake, non-specific to drug treatment. There was no interaction of group by phase [F (2, 36) = 2.085, p > 0.05], again, suggesting that quinpirole did not have a specific effect on ethanol intake.

Raclopride

Data for mean ethanol intake (g/kg) within a 22-hr continuous access paradigm, across baseline, treatment and post-treatment period are presented in Figure 5. Ethanol intake (g/kg) was not significantly different between groups [F (1, 18) = 0.074, p > 0.05] or across phases [F (2, 36) = 1.141, p > 0.05] thus, not supporting the effects of raclopride observed in Experiment 1. A significant interaction between drug group and phase period [F (2 36) = 4.003, p < 0.05] was further examined with a test of simple effects. The analysis reflected the finding that raclopride-treated rats drank significantly more ethanol across phases than controls [F (2, 36) = 3.789, p < 0.05]. Simple comparisons confirmed that ethanol intake during the treatment phase was significantly increased compared to baseline [F (1, 9) = 9.734, p < 0.05]. Ethanol intake (g/kg) during treatment and post-treatment did not significantly differ [F (1, 9) = 0.346, p > 0.05] reflecting that the level of ethanol intake was maintained during the post-treatment period.

Similarly, there was no main effect of group [F (1, 18) = 0.637, p > 0.05] or phase [F (2, 36) = 1.337, p > 0.05] as measured by the volume of ethanol (mls) ingested. There was a significant drug by phase interaction [F (2 36) = 3.482, p < 0.05](see Figure 6). Analysis of simple effects followed by simple comparisons, again indicated that raclopride increased ethanol intake (mls) during the treatment phase [F (1, 9) = 9.133, p < 0.05].

Ethanol preference ratios were not significantly different between the groups [F (1, 18) = 0.431, p > 0.05] or across phases [F (2, 36) = 1.527, p > 0.05] (see Figure 7). A significant interaction was obtained between group and phase
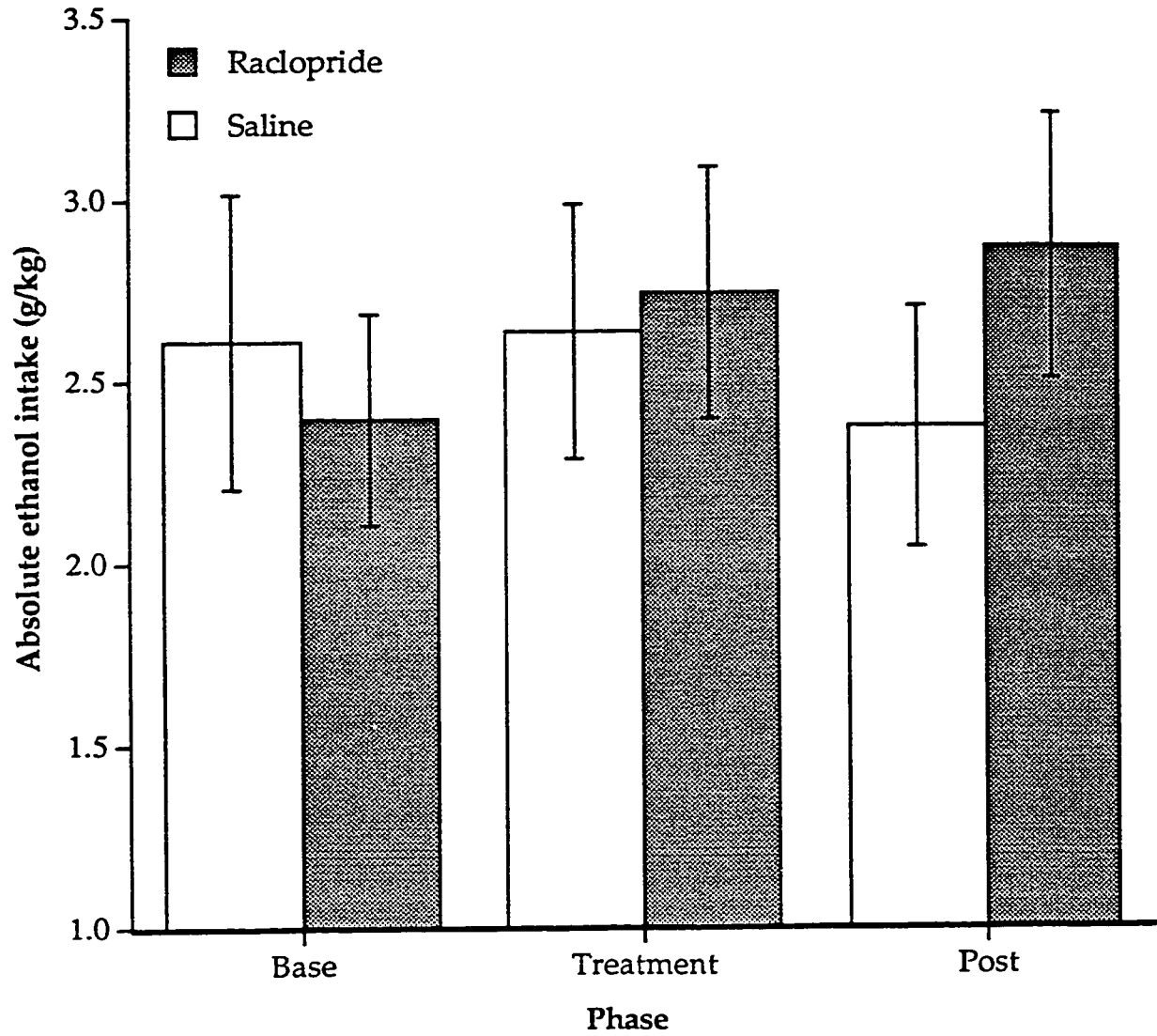
Figure 5 . The effect of raclopride on mean ethanol intake across 4-day phases. Absolute ethanol intake (g/kg) during continuous access is presented. Vertical bars represent S.E.M.

Figure 6. Effect of raclopride on mean ethanol intake across 4-day phases. Mean ethanol intake (mls) presented. Vertical lines represent S.E.M.
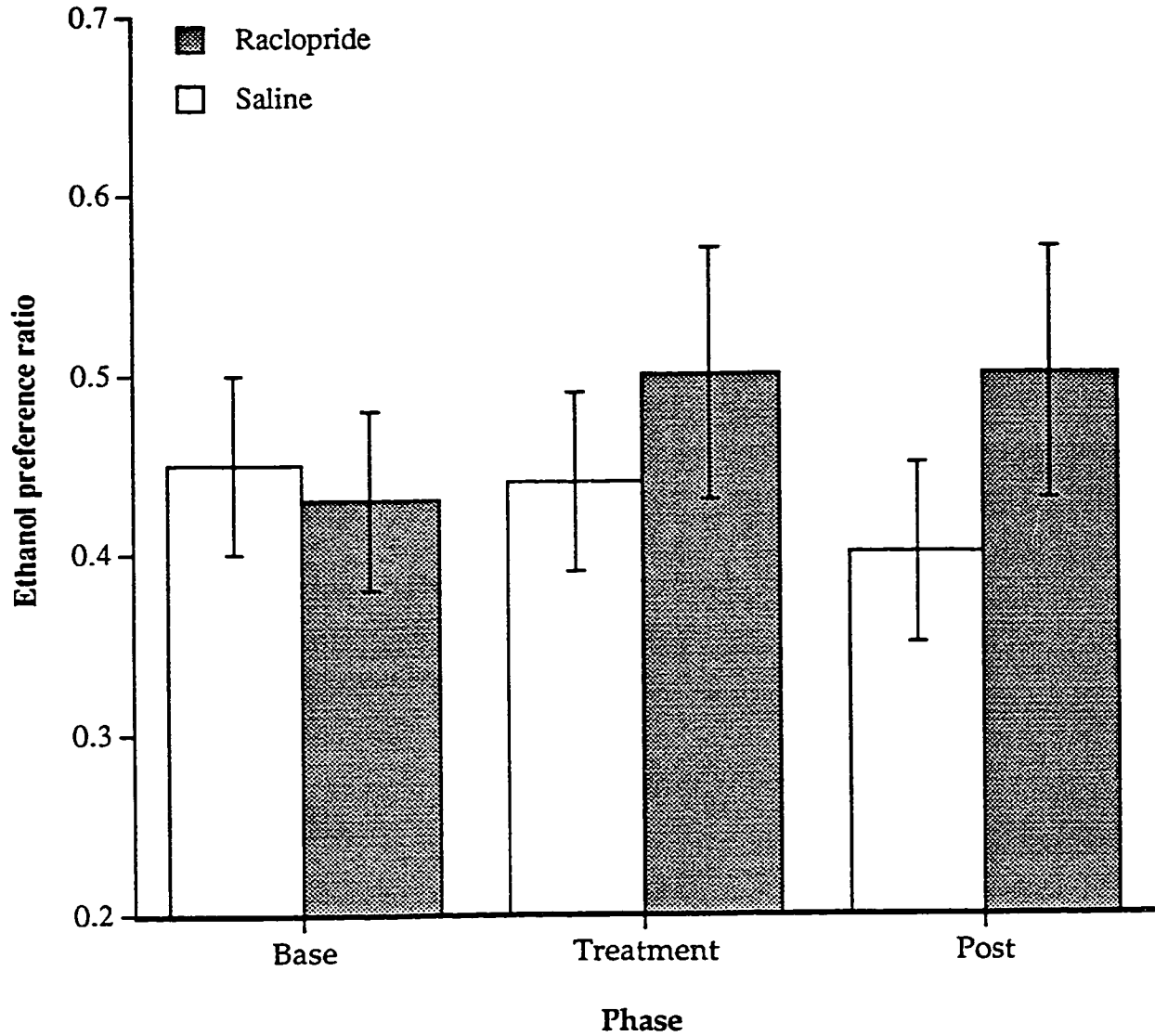
<u>Figure 7</u>   The effect of raclopride on preference for a 10% ethanol
solution over water. Preference (EtOH mls/Total fluid)
presented. Vertical lines represent S.E.M.

[F (2, 36) = 3.482, p < 0.05] reflecting the finding that raclopride-treated rats preferred ethanol to water during the treatment phase [F (1, 9) = 10.41, p < 0.05]. Accordingly, water intake (mls) did not significantly differ between groups [F (1, 18) = 0.431, p > 0.05] or phase [F (2, 36) = 1.527, p > 0.05]. Analysis of the significant interaction between groups and phase [F (2 36) = 3.482, p < 0.05] revealed that saline-treated animals increased their water intake during post-treatment as compared to baseline [F (1, 9) = 7.577, p < 0.05] (see Figure 8).

Total fluid intake (mls) was not significantly different between drug groups [F (1, 18) = 0.056 p > 0.05]. There was a main effect of phase [F (2, 36) = 3.712, p < 0.001] reflecting differences in total fluid intake across phases (Figure 9). Post hoc analysis revealed an increased total fluid intake during post-treatment when compared to baseline (p < 0.05). This finding probably reflected the additive effects of a significant increase in water intake for the control group and the slight but non-significant increase in ethanol intake for the raclopride group. There was no interaction of group by phase [F (2, 36) = 0.681, p > 0.05] suggesting that raclopride did not have a general effect on fluid consumption.

DISCUSSION

The goal of Experiment 2 was to assess the effects of the mixed D2/D3 agonist quinpirole and the mixed D2/D3 antagonist raclopride on ethanol and water consumption within a 22-hr continuous access paradigm. The analysis of the quinpirole data of experiment 2 indicated that the D2/D3 agonist did not have a significant effect on ethanol intake (g/kg and mls) when examined within a continuous access paradigm. Water intake was not significantly different between the quinpirole and saline control group. However, total fluid intake for
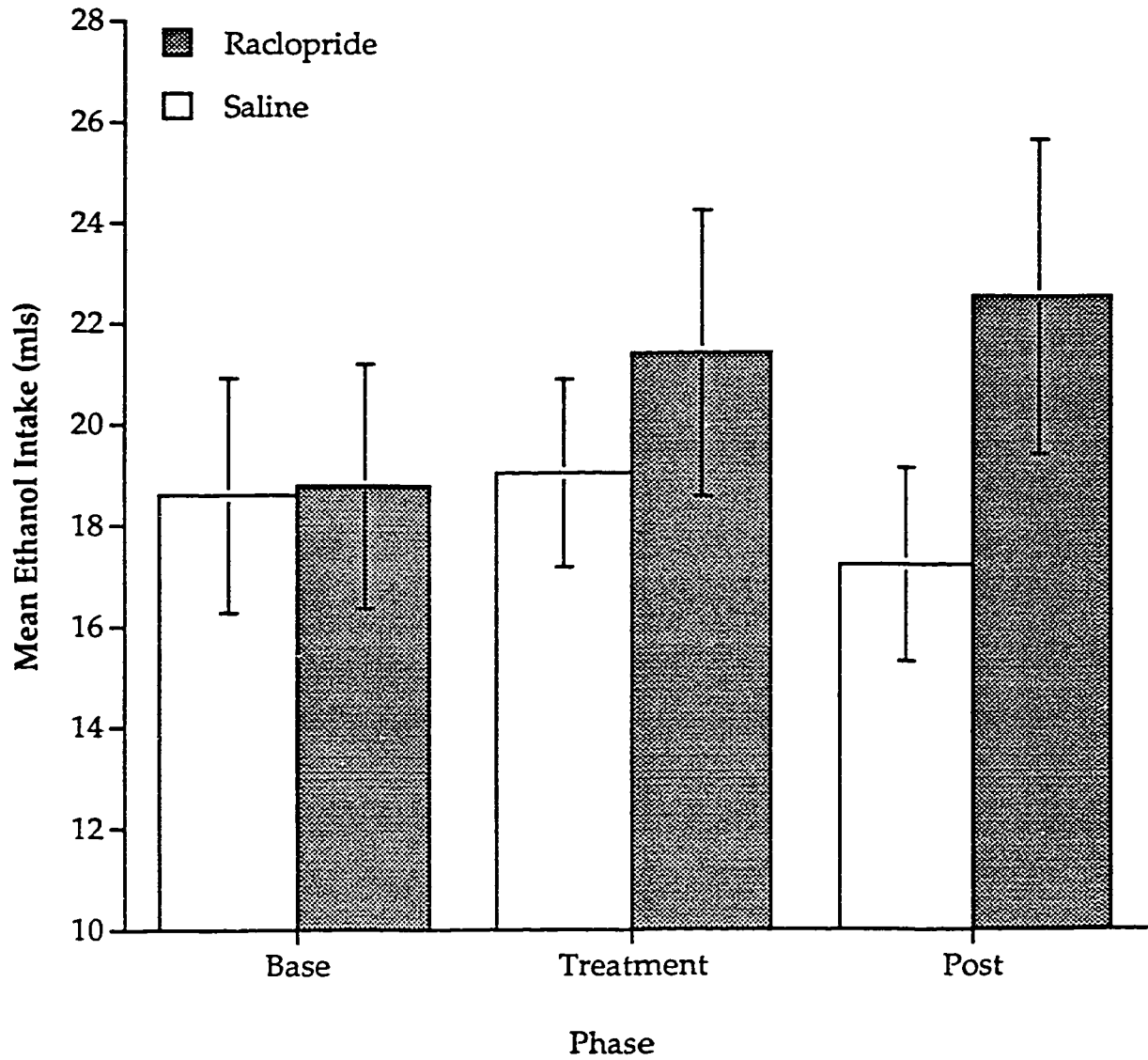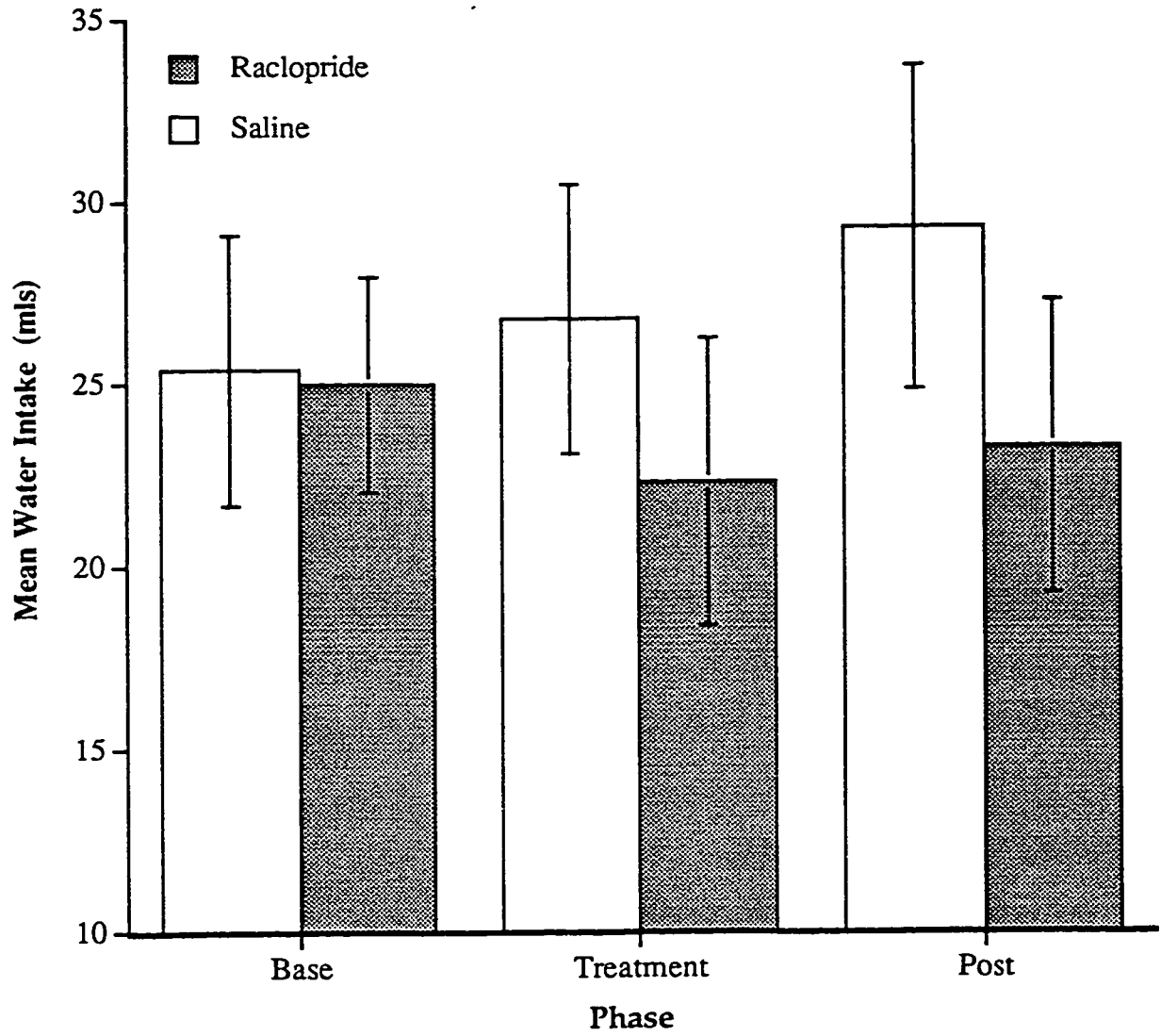
Figure 8. Effect of raclopride on water intake across 4-day phases. Mean water intake (mls) represented. Vertical lines represent S.E.M.
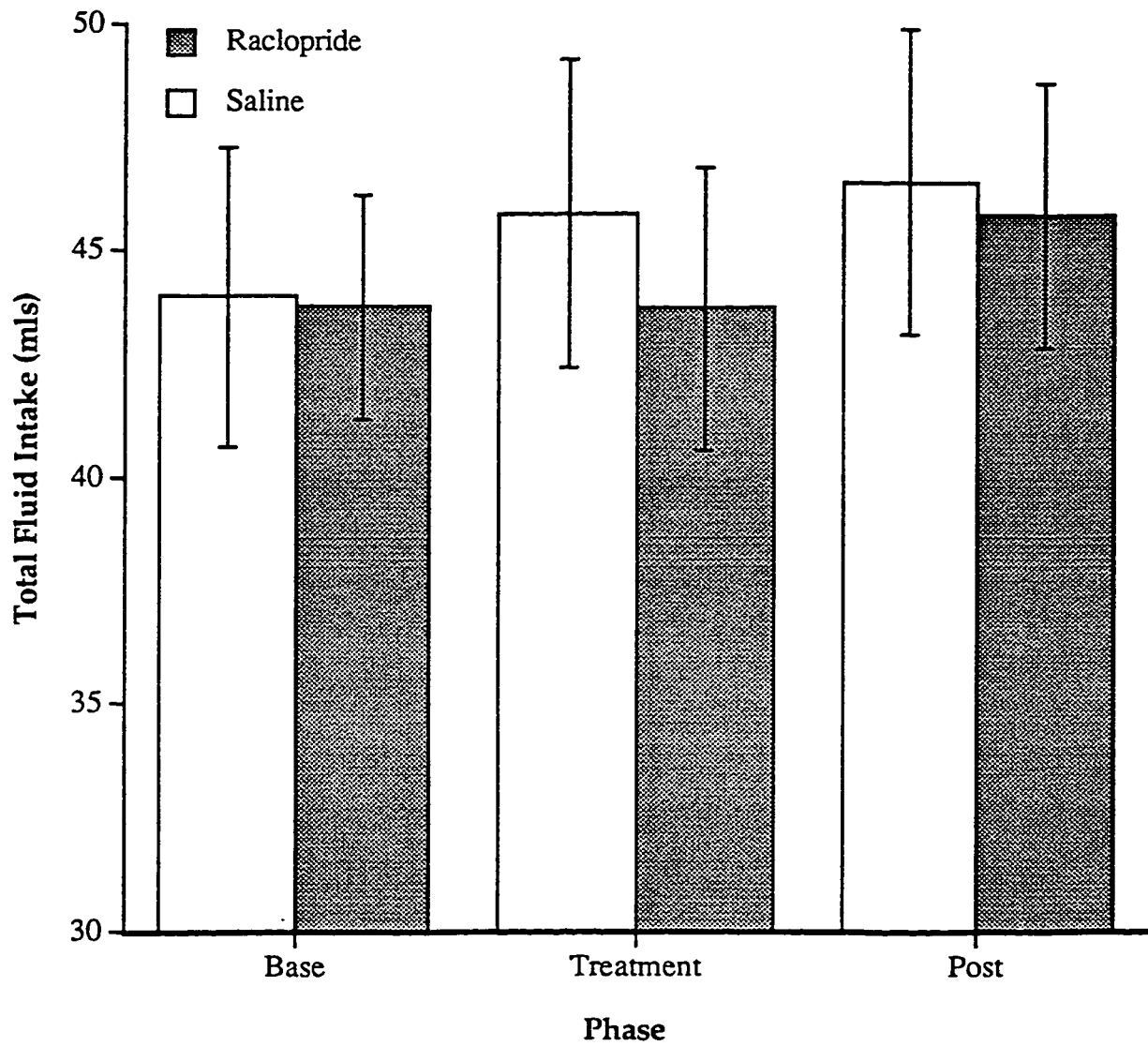
Figure 9. Effect of raclopride on total fluid intake across 4-day phases. Total fluid intake (mls) represented. Vertical lines represent S.E.M.

both groups increased during treatment and was maintained post-treatment indicating a general fluctuation in fluid intake, non-specific to drug treatment.

Results of the raclopride analysis revealed that raclopride-treated rats significantly increased their ethanol intake (g/kg and mls) during treatment but did not significantly decrease their water and total fluid intake. Ethanol preference ratios similarly demonstrated that the raclopride group preferred ethanol to water during the treatment phase. These results suggested that the increase in ethanol intake was not due to a general increase in fluid intake but to a specific effect on ethanol.

GENERAL DISCUSSION

The purpose of this thesis was to investigate the role of central dopamine D2 and D3 receptors in the mediation of the reinforcing properties of voluntary ethanol intake within a limited and a continuous access paradigm. Specifically, the goal was to elucidate whether decreases in ethanol consumption after dopaminergic drug administration were therefore due to a dopamine-mediated reduction in reinforcement or rather to some other non-specific drug effects, such as locomotor impairment. Furthermore, it was important to determine whether the direction of the obtained effects were consistent across the limited and continuous access paradigms.

Experiment 1 was designed to assess the effects of raclopride and quinpirole on ethanol consumption within a limited access paradigm. The findings of experiment 1 revealed that raclopride significantly decreased ethanol intake during the 1-hr limited access period. The observation that the antagonist, raclopride decreased ethanol intake in experiment 1 supports previous findings (Pfeffer and Samson, 1986; Rassnick et al. 1992) and endorses the notion that dopamine may play a role in mediating ethanol reinforcement. On the other hand, quinpirole failed to alter ethanol intake within this same access paradigm and time period. The results indicated that it was a drug group X phase period interaction which influenced ethanol intake. However, further analysis of the interaction did not identify any significant effects within levels of the drug or phase factor. Thus, differences in ethanol intake across phase were probably the consequence of increased variability among subjects as opposed to a clear drug effect. The finding that quinpirole failed to alter ethanol intake in Experiment 1 is both contrary (Dyr et al. 1993) as well as consistent (Linesman, 1990) with previous reports. The fact that quinpirole did not produce a decrease in ethanol

intake suggests that manipulations which activate D2/D3 receptors may not produce an effect on ethanol consumption in non-selected rats. Thus, on the basis of Experiment 1, it cannot be argued that activity in dopamine D2/D3 receptors may mediate the reinforcing properties of ethanol consumption.

In order to assess whether the effects obtained within a limited access paradigm are comparable and/or generalizable to a continuous access paradigm, rats were given 22-hr access to ethanol. Contrary to the findings of Experiment 1, the results of Experiment 2 demonstrated that raclopride increased ethanol intake and preference when examined within a continuous access paradigm. Water intake levels of the raclopride-treated group and the control group were not significantly different. Thus, the increase in ethanol intake of the raclopride group in Experiment 2 appears to be specific to ethanol and not due to a general effect on consummatory behavior. However, the increase in ethanol intake after raclopride administration conflicts with most reports on the effects of systemically administered dopamine antagonists on ethanol intake (e.g.: Sylvestre et al. 1996; Rassnick et al. 1992).

Consistent with the findings obtained in Experiment 1, Experiment 2 showed that quinpirole did not produce any significant effects on ethanol intake and/or preference within the continuous access paradigm. Total fluid intake was increased for both the quinpirole and control groups across phases suggesting that this effect was not specific to the drug treatment.

A comparison of the findings obtained from Experiment 1 and Experiment 2 reveal both a consistent effect of quinpirole and a contradictory effect of raclopride on ethanol consumption across access paradigms. The lack of effect of quinpirole as well a raclopride's opposite effect across paradigms raise questions about the notion that dopamine D2/D3 receptors are critical in mediating

ethanol reinforcement. Quinpirole administration failed to specifically attenuate ethanol intake within a continuous access paradigm and more importantly within a limited access paradigm. It has been suggested that the effect of dopaminergic agents on ethanol consumption are best examined when using a limited access paradigm (Pfeffer and Samson, 1986). Thus, the observation that quinpirole administration did not affect ethanol intake in Experiment 2 is consistent with several reports indicating that testing the effect of DA agents on ethanol intake within continuous access does not produce positive findings (Brown et al. 1982; Goodwin et al. 1996). The failure to observe an effect of quinpirole on ethanol intake within a continuous access paradigm, confirmed the findings of Experiment 1. However, the fact that quinpirole did not have a significant effect on ethanol intake does not extend support for the notion that activating DA receptors should decrease ethanol intake in rodents (e.g. Dyr et al. 1993) as well as alcohol-preferring C57 mice (George et al. 1995). Therefore, the findings on the 0.1 mg/kg dose of quinpirole do not support the involvement of D2/D3 receptors in the reinforcement of oral ethanol self-administration within a 22-hr continuous access paradigm.

Of particular interest was the finding that ethanol intake in rats even within the limited access paradigm was not directly influenced by the administration of the mixed D2/D3 agonist quinpirole. The failure of quinpirole to produce a reduction in ethanol intake within the limited access paradigm is at odds with previous findings (e.g., Dyr et al. 1993) which suggested the putative involvement of dopamine D2 and D3 receptors in oral ethanol intake. The discrepancy between the results obtained in Experiment 1 and in the study by Dyr et al., may be linked to differences in the methodology used in the two studies. In particular, Dyr et al. reported that they observed reductions in ethanol intake, 4-hr after the administration of several doses (0.08 - 0.25 mg/kg)

of quinpirole, in high alcohol-drinking (HAD) female rats. The use of genetically selected HAD rats over a 4-hr time period makes direct comparisons with other limited access studies difficult. These methodological differences notwithstanding, a larger problem inherent in the Dyr et al. (1993) study is that insufficient information is provided to the reader to allow a detailed analysis of the results. The dose-related changes in ethanol consumption seem to be attributable only to the 0.25 mg/kg dose at the end of the 4-hr period. In addition, upon further examination of the results provided by Dyr et al. it is apparent that all doses of quinpirole similarly failed to produce a significant effect at the 1-hr mark of the 4-hr period. Interestingly, ethanol consumption after the 0.25 mg/kg dose of quinpirole actually appears to increase from 2 ml to 5 ml over the 4-hr period, questioning the finding that the reduction persisted over the entire testing period.

The fact that quinpirole at a 0.1 mg/kg dose did not attenuate ethanol consumption within a 1-hr access period replicates a previous report in the literature (Linesman, 1990) and suggests that pharmacological activation of the D2/D3 receptors when examined shortly after administration, may not influence ethanol intake. Thus, it appears that the failure of quinpirole at a dose of 0.1 mg/kg to reduce the amount of ethanol consumed within a 1-hr limited access period in Experiment 1 actually both confirms and extends the reports in the literature by Linesman, (1990) as well as those obtained by Dyr et al. (1993). The possibility exists that a dose larger than 0.1 mg/kg of quinpirole may be required to observe a reduction in ethanol consumption. However, it has previously been shown that a 0.3 mg/kg dose of quinpirole (Linesman, 1990) was not effective in reducing ethanol intake within a 1-hr limited access period.

A comparison of raclopride's effect in Experiment 1 and 2 could be interpreted as support for the notion that D2/D3 receptors play a role in mediating ethanol reinforcement and subsequent intake. In Experiment 1, the finding of decreased ethanol intake observed after raclopride administration suggested that D2/D3 receptor blockade was effective in reducing ethanol intake through a dopamine-mediated reinforcement mechanism. However, this explanation was rendered less plausible when gross observations of the rats post-injection were incorporated into the analysis. Observation of animals for a 2-hr period post-drug treatment, indicated that raclopride-treated animals were ataxic and therefore were not able to consume ethanol. Thus, since animals did not drink because raclopride presumably restricted their ability to move, it follows logically that the decrease in ethanol intake was not a result of an observed decrease in dopamine-mediated ethanol reinforcement but rather because of ataxia. Furthermore, in Experiment 2 raclopride significantly increased ethanol intake (g/kg and mls) as opposed to the decrease observed in Experiment 1. The fact that water intake was not affected by raclopride in Experiment 2 argues against previous reports suggesting that raclopride administration had a general effect on consummatory behavior (Sylvestre et al. 1996).

As mentioned above, initially, the results of raclopride administration in Experiment 1 and 2, could have been interpreted as support for the hypothesis of a dopaminergic mechanism in ethanol reinforcement. However, while it is true that ethanol intake was modified by raclopride administration in both experiments and thus pointing to the possible involvement of dopamine, this interpretation is complicated because the obtained effects in Experiment 2 are opposite to the results of the first experiment. Furthermore, the increased ethanol intake after raclopride administration observed in Experiment 2 does not support previous findings that systemic injections of dopamine antagonists

resulted in decreased ethanol intake (Fuchs et al. 1984; Pfeffer & Samson, 1985;1988).

One possible explanation for the diametrically opposite effects obtained in the two experiments may be that measuring the behavioral effects of dopamine receptor blockade on ethanol intake after an extended period post-drug administration as in Experiment 2, allows for the sedative effects of raclopride to wear off and for a rebound effect on intake to occur. It has been established that rodents will adjust their drug intake levels in a manner that keeps the level of drug on which they are maintained at a constant level (e.g.: Amit and Corcoran, 1975). On the other hand, several researchers (Gill et al. 1988; Goodwin et al. 1996) have not been able to demonstrate the occurrence of this putative compensation phenomenon within a single 24-hr drinking session. While Gill and colleagues (1988) examined the effect of a 5-HT agent and observed general decreases on consummatory behavior, their finding suggests that a compensation interpretation is unlikely.

Another finding which warrants consideration with respect to the compensation interpretation of the present data is the maintained level of increased drinking following the termination of drug treatment. In Experiment 2, ethanol consumption remained elevated throughout the four day post-treatment period following treatment with 0.5 mg/kg raclopride. It seems reasonable to expect that if raclopride was affecting the reinforcing properties of ethanol during the treatment period, once removed, animals' ethanol intake should rapidly return to baseline levels. This increase in consumption during the post-treatment period cannot be due to continued DA receptor blockade, since raclopride is quickly metabolized and no trace remains in plasma 24 hrs after drug administration (Ahlenius, Ericson, Hogberg & Wijkstrom, 1991). Thus, the continued increase in consumption is inconsistent with a compensation

interpretation of the data. The absence of recovery of pre-treatment drinking

levels following the drug treatment period suggests that the alcohol-experienced

animals in Experiment 2, may have maintained their ethanol intake for the

pharmacological effects of ethanol. Nonetheless, it is important to note, that

while the present findings suggested that manipulations which produced specific

effects on ethanol intake did so as a function of changes in motor capacity, the

precise mechanisms mediating these changes remain uncertain.

DIRECTIONS FOR FUTURE RESEARCH

When future research is undertaken it would be both interesting and

useful to attempt to further differentiate between the effects of raclopride on the

intake of ethanol and motor capacity. This is of particular importance because

raclopride did not have the same effect on ethanol intake in Experiment 1 and 2.

It is possible that the 0.5 mg/kg dose of raclopride may be high and not allow for

the dissociation between a dopamine mediated reduction in ethanol

reinforcement as compared to motor suppression. Studies on the behavioral

profile of raclopride (Hillegaart and Ahlenius, 1987) have demonstrated that 0.5

mg/kg is the threshold dose for the emergence of raclopride's motor suppressant

effects. Reductions in spontaneous locomotor activity with a 0.5 mg/kg dose are

reported to occur within 15 minutes, peak 1-2 hr later and disappear by the 4th

hour after drug administration. However, pharmacological studies on the

neurobiological substrates underlying ethanol consumption, such as the study by

Sylvestre et al. (1996), have used comparable and even a higher dose (1.0 mg/kg)

than the 0.5 mg/kg dose employed in Experiments 1 and 2, yet these

investigators failed to detect the occurrence of extrapyramidal motor effects

(EPS). Thus, to further elucidate the separation between raclopride's

motivational effect and EPS effects, it would be useful to examine the effect of a 0.5 mg/kg dose on ethanol intake within a continuous operant paradigm. Furthermore, since a different effect of 0.5 mg/kg raclopride on ethanol consumption was observed within a limited and continuous access, it would be useful to examine the effect of several lower doses of raclopride on ethanol intake and motor capacity. In addition, it would be useful to study the effects of raclopride on the consumption of a pharmacologically inert, flavored solution such as saccharin, to further elucidate the specificity of raclopride's effect on fluid consumption.

CONCLUSION

The evidence supporting a dopaminergic mechanism of reinforcement in the consumption of ethanol is equivocal at best. It appears that positive effects of dopaminergic agents on ethanol intake occur within very specific constraints. As pointed out by Rassnick et al. 1993, it is curious that manipulations which are effective in reducing ethanol intake tend to occur more frequently with the use of operant procedures (lever press) within a limited access period and typically fail when the model of human oral consumption is used.

In conclusion, the experiments conducted within the framework of this thesis, found no evidence that the mixed D2/D3 agonist quinpirole could attenuate ethanol intake, regardless of the access paradigm employed. On the other hand, the mixed D2/D3 antagonist raclopride produced a decrease in ethanol intake within a limited access paradigm that appeared to be mediated through a suppression of locomotor capacity. When continuous access to ethanol was instituted, raclopride produced an increase in ethanol intake that was maintained during post-treatment, raising some questions about the notion that

blocking DA transmission would attenuate ethanol reinforced drinking. Taken together, the data obtained in this thesis do not provide a basis for supporting the primary notion of the involvement of dopamine D2 and D3 receptors in mediating ethanol self-administration. However, these findings do not preclude the possibility that activation of dopamine receptors may in part, have a role in the mediation of voluntary ethanol consumption.

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Appendix A: Analysis of Variance Summary Tables

Experiment 1: ANOVA Summary Table for Ethanol Intake (g/kg)

QUINPIROLE

Source	SS	df	MS	F	p
Drug (D)	0.094	1	0.094	0.627	0.4389
Error	2.693	18	0.15		
Phase (P)	0.020	2	0.01	0.820	0.4484
P x D	0.098	2	0.049	4.013	0.0267
Error	0.439	36	0.012		

Simple Effects

Source	MSn	dfn	dfe	Mse	F	p
D at Base	0.003	1	18	0.060	0.056	0.815
D at Treatment	0.145	1	18	0.067	2.173	0.158
D at Post	0.043	1	18	0.047	0.909	0.353
Phase at Quinpirole	0.026	2	36	0.012	2.142	0.132
Phase at Saline	0.033	2	36	0.012	2.691	0.081

RACLOPRIDE

Source	SS	df	MS	F	p
Drug (D)	0.921	1	0.921	10.465	0.0046
Error	1.594	18	0.088		
Phase (P)	1.759	2	0.880	54.612	0.0001
P x D	1.198	2	0.599	37.198	0.0001
Error	0.580	36	0.016		

Simple Effects

Source	MSn	dfn	dfe	Mse	F	p
D at Base	0.005	1	18	0.049	0.100	0.755
D at Treatment	2.093	1	18	0.018	116.564	0.001
D at Post	0.021	1	18	0.053	0.396	0.537
Phase at Raclopride	1.446	2	36	0.016	89.806	0.001
Phase at Saline	0.032	2	36	0.016	2.004	0.150

Experiment 2: ANOVA Summary Table for Ethanol Intake (mls)

QUINPIROLE

Source	SS	df	MS	F	p
Drug (D)	188.151	1	188.510	1.253	0.2778
Error	2703.952	18	150.220		
Phase (P)	31.777	2	15.889	1.220	0.3072
P x D	63.215	2	31.607	2.426	0.1027
Error	468.967	36	13.027		

RACLOPRIDE

Source	SS	df	MS	F	p
Drug (D)	102.704	1	102.704	0.637	0.4353
Error	2904.004	18	161.334		
Phase (P)	25.390	2	12.695	1.337	0.2753
P x D	66.102	2	33.051	3.482	0.0415
Error	341.758	36	9.493		

Simple Effects

Source	MSn	dfn	dfe	Mse	F	p
D at Base	0.153	1	18	55.970	0.003	0.959
D at Treatment	28.203	1	18	57.255	0.493	0.492
D at Post	140.450	1	18	67.096	2.093	0.165
Phase at Raclopride	36.627	2	36	9.493	3.858	0.030
Phase at Saline	9.119	2	36	9.493	0.961	0.392

Experiment 2: ANOVA Summary Table for Ethanol Intake (g/kg)

QUINPIROLE

Source	SS	df	MS	F	p
Drug (D)	1.473	1	1.473	0.417	0.5268
Error	63.637	18	3.535		
Phase (P)	0.495	2	0.248	1.123	0.3366
P x D	1.207	2	0.604	2.737	0.0783
Error	7.940	36	0.221		

RACLOPRIDE

Source	SS	df	MS	F	p
Drug (D)	0.250	1	0.250	0.074	0.7888
Error	60.759	18	3.375		
Phase (P)	0.358	2	0.179	1.141	0.3309
P x D	1.257	2	0.628	4.003	0.0269
Error	5.652	36	0.157		

Simple Effects

Source	MSn	dfn	dfe	Mse	F	p
D at Base	0.229	1	18	1.253	0.183	0.674
D at Treatment	0.057	1	18	1.221	0.047	0.831
D at Post	1.220	1	18	1.215	1.004	0.330
Phase at Raclopride	0.595	2	36	0.157	3.789	0.032
Phase at Saline	0.213	2	36	0.157	1.355	0.271

Experiment 2: ANOVA Summary Table for Water Intake (mls)

QUINPIROLE

Source	SS	df	MS	F	p
Drug (D)	114.264	1	114.264	0.325	0.5754
Error	6320.623	18	351.146		
Phase (P)	81.484	2	40.742	2.705	0.0805
P x D	24.556	2	12.278	0.815	0.4506
Error	542.26	36	15.063		

RACLOPRIDE

Source	SS	df	MS	F	p
Drug (D)	198.017	1	198.017	0.475	0.4994
Error	7499.604	18	416.645		
Phase (P)	30.431	2	15.216	1.432	0.2520
P x D	84.415	2	42.207	3.973	0.0276
Error	382.446	36	10.623		

Simple Effects

Source	MSn	dfn	dfe	Mse	F	p
D at Base	0.800	1	18	112.616	0.007	0.934
D at Treatment	100.128	1	18	145.675	0.687	0.418
D at Post	181.503	1	18	179.600	1.011	0.328
Phase at Raclopride	18.390	2	36	10.623	1.731	0.192
Phase at Saline	39.033	2	36	10.623	3.674	0.035

Experiment 2: ANOVA Summary Table for Total Fluid Intake (mls)

QUINPIROLE

Source	SS	df	MS	F	p
Drug (D)	9.600	1	9.600	0.042	0.840
Error	4116.092	18	228.672		
Phase (P)	190.908	2	95.454	11.034	0.0002
P x D	36.075	2	18.038	2.085	0.1391
Error	311.433	36	8.651		

RACLOPRIDE

Source	SS	df	MS	F	p
Drug (D)	15.504	1	15.504	0.056	0.8160
Error	5004.158	18	278.009		
Phase (P)	51.377	2	25.689	3.712	0.0342
P x D	9.427	2	4.714	0.681	0.5125
Error	249.154	36	6.921		

Experiment 2: ANOVA Summary Table for Ethanol Preference Ratio (EtOHmls/Total mls)

QUINPIROLE

Source	SS	df	MS	F	p
Drug (D)	0.036	1	0.036	0.457	0.5076
Error	1.399	18	0.078		
Phase (P)	0.003	2	0.002	0.295	0.7465
P x D	0.015	2	0.007	1.426	0.2534
Error	0.186	36	0.005		

RACLOPRIDE

Source	SS	df	MS	F	p
Drug (D)	0.039	1	0.039	0.431	0.5199
Error	1.631	18	0.091		
Phase (P)	0.011	2	0.005	1.527	0.2309
P x D	0.039	2	0.019	5.587	0.0077
Error	0.125	36	0.003		

Simple Effects

Source	MSn	dfn	dfe	Mse	F	p
D at Base	0.001	1	18	0.029	0.050	0.825
D at Treatment	0.021	1	18	0.033	0.647	0.432
D at Post	0.055	1	18	0.036	1.523	0.233
Phase at Raclopride	0.018	2	36	0.003	5.062	0.012
Phase at Saline	0.007	2	36	0.003	2.052	0.143