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Isotretinoin (13-*cis*-retinoic acid) alters learning and memory, but not anxiety-like behavior, in the adult rat

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ABSTRACT

Isotretinoin (ISO, 13-*cis*-retinoic acid) is commonly prescribed as *Accutane* for the treatment of acne. ISO is a known teratogen and the physical side effects of the drug have been well documented. However, possible psychological risks associated with the drug have yet to be determined. Retinoid receptors are abundant in the striatum and hippocampus, brain structures involved in implicit and explicit memory processes, respectively. The current study examined whether ISO influenced implicit or explicit memory processes using a two-stage radial-arm maze (RAM) task. The two stages were identical, except for the method of presenting arm choices to the rats: one at a time (Stage 1) or in pairs (Stage 2). Male rats (n=12/group) were tested on both stages of the RAM during chronic oral treatment with ISO (0, 5, 10, or 15 mg/kg/day). Performance indicated that ISO impaired explicit memory in Stage 2, but retention tests one month after ISO exposure ended, indicated recovery from this explicit memory impairment and evidence of enhanced implicit memory in the 10 mg and 15 mg ISO rats. These data indicate extensive, enduring memory effects from oral ISO treatment at doses likely to produce serum levels within the range typically used to treat acne in humans.

Vitamin A and its derivatives, the retinoids, are essential for the development and maintenance of body tissues and central nervous system function (Lane and Bailey, 2005; Maden, 2007). Isotretinoin (ISO, 13-*cis*-retinoic acid) is a naturally-occurring retinoid that is also the active ingredient in the acne medication *Accutane* (Hoffman-LaRoche, Nutley, NJ). First synthesized in 1955, ISO has proven to be highly effective in treating dermatological diseases; however, prescription use of the drug has been rigidly controlled ever since its 1982 FDA approval, mainly because it has been associated with severe teratogenic effects, but also because it has been associated with a variety of physical side effects (e.g., dry/peeling skin, muscle pain, vomiting, headache, fatigue, intracranial hypertension, tremors or seizures, numbness or paralysis, and blurred or double vision) in users (Roche, 2002). Not surprisingly, because ISO is a derivative of vitamin A, its side effects are similar to the symptoms experienced with vitamin A toxicity, or hypervitaminosis A (O'Donnell, 2003, 2004). Due to the many side effects associated with ISO's use, it has never been approved as a first-line acne treatment, but only for cases of severe recalcitrant nodular acne, a severe form of acne generally considered to be resistant to standard treatments (e.g., oral antibiotics). However, in recent years, the use of ISO for less severe cases of acne has reportedly been on the rise with recent statistics showing that half of all prescriptions are written for patients not diagnosed with severe acne (Bremner and McCaffery, 2008). *Accutane* use more than tripled during the previous decade with nearly 2 million prescriptions being filled in the U.S. alone during the year 2000 (Roche, 2002).

While many physical side effects and risks have been associated with prenatal and adult exposure to ISO, possible psychological risks associated with adult use have yet to be fully determined. Reports of suicidal ideation, depression, personality changes, memory loss, violence, and aggression in patients taking ISO have raised concerns in recent years about serious psychological effects associated with the drug (O'Donnell, 2003). While studies investigating potential psychological risks of ISO have been equivocal (Bremner et al., 2005; Chia et al., 2005; Cohen et al., 2007; Ferguson et al., 2005; Hull and D'Arcy, 2003; Magin et al., 2005; Marqueling and Zane, 2005; O'Reilly et al., 2006), ISO's list of contraindications and warnings has continually increased over the years to now include the possibility of acute or chronic psychiatric disorders, including sadness, depression, irritability, increased aggression, loss of concentration, loss of appetite, and suicide-related behavior (Roche, 2002).

The distribution of retinoid receptors in the cortex, hippocampus, and dopamine (DA)-innervated areas such as the striatum (caudate/putamen), nucleus accumbens, and the olfactory tubercle (Krezel et al., 1999; Zetterstrom et al., 1994, 1999) suggests that several areas of the adult brain respond to retinoids, and the functional processes of these brain regions may be influenced by manipulation of retinoid availability (Lane and Bailey, 2005). Thus, a major focus of research investigating the role of retinoids in the central nervous system has

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been their interaction with DA systems (Samad et al., 1997; Krezel et al., 1998). These studies have generally supported the hypothesis that regulation of DA receptors by retinoid receptor activation influences the expression of DA in the central nervous system (Samad et al., 1997). For instance, Krezel et al. (1998) found that the expression of D_1 and D_2 receptors was reduced in the ventral striatum of adult retinoid receptor mutant mice. Functionally, these mice displayed impaired locomotion, decreased coordination, and a reduction in DA signaling in the mesolimbic system compared to normal mice. Response to cocaine, which normally increases locomotion by increasing DA signaling in the mesolimbic system, was also blunted. While these changes may be due to alterations that take place during embryonic development, research suggests that retinoids still play an important, although perhaps different, role in the mature brain (see Lane and Bailey, 2005 for review). Together, these findings highlight the involvement of retinoid receptors in the regulation of brain DA systems known to be involved in regulation of movement, reward/reinforcement, and implicit forms of learning and memory.

In recent years, retinoids have also been demonstrated to have an important role in the learning and memory systems of the hippocampus. In retinoid receptor knockout mice, a decrease in hippocampal long-term potentiation (LTP) and long-term depression (LTD), with a concomitant impairment in spatial learning and memory, has been demonstrated (Chiang et al., 1998). Furthermore, animals fed a vitamin A deficient (VAD) diet have been shown to have impaired hippocampal LTP/LTD, reduced size of CA1 neurons, a reduction in retinoid receptor mRNAs, and reduced neuronal protein RC3, a protein involved in the functional plasticity of hippocampal synapses during relational memory processing (Misner et al., 2001; Cocco et al., 2002; Etchamendy et al., 2003). Functionally, these VAD animals have been shown to be impaired in hippocampal-dependent learning and memory tasks (Cocco et al., 2002; Etchamendy et al., 2003). Further evidence of a role for retinoids in learning and memory processes comes from the demonstration that many of the VAD-induced impairments mentioned above are reversible when animals are returned to a vitamin A sufficient diet (Misner et al., 2001; Etchamendy et al., 2003). Given that both DA and acetylcholine (ACh) have been shown to play important roles in hippocampal LTP/LTD (Kusuki et al., 1997; Otmakhova and Lisman, 1998; Disterhoft and Oh, 2003) and have been shown to be impaired in VAD mice (Cocco et al., 2002) and retinoid receptor deficient mice (Krezel et al., 1998), these neurotransmitter systems have been implicated in these hippocampal impairments. While it is important to note that differences in the route of drug administration, species, and age, will almost certainly result in differences in RA metabolism and signaling mechanisms, the literature suggests that, regardless of these differences, alterations in retinoic acid influence hippocampal learning and memory systems.

A recent study by Etchamendy et al. (2001) is of particular relevance to understanding the role of retinoid receptors in learning and memory processes. This study used a two-stage radial-arm maze (RAM) paradigm that distinguished between the expression of implicit and explicit memory. During Stage 1 of this task, individual arms of the RAM were presented repeatedly to rats one by one (i.e., successive go/no-go discrimination), either baited or unbaited, until they learned to distinguish which arms contained food rewards. Successful discrimination was indicated by shorter latencies to enter baited than unbaited arms. Stage 2 of this task used the same go/no-go reward contingencies learned in Stage 1, but grouped the single arm presentations into adjacent pairs that would be presented concurrently. Animals were thus presented with an explicit choice between one baited (positive) arm and one unbaited (negative) arm.

The uniqueness of this two-stage paradigm is that it is thought to provide a test of both implicit and explicit learning and memory processes within the same testing apparatus (Marighetto et al., 1999, 2000). The first stage of this task assesses implicit memory, which facilitates particular routines that do not require relational comparisons, and is heavily reliant on striatal mechanisms. Clinical and behavioral evidence suggests that the striatum is centrally involved in the stimulus–response associations and procedural learning that leads to habit formation and the improved performance of routine behaviors (Jog et al., 1999; Packard and Knowlton, 2002; Poldrack and Packard, 2003; Squire, 1998; Teng et al., 2000). The second stage, which requires animals to make a relational/concurrent discrimination, tests explicit memory (Etchamendy et al., 2001; Touzani et al., 2003). Explicit memory is required for flexibility in comparing and contrasting items in memory as well as the capacity to support inferential use of memories in novel situations. This type of memory requires the hippocampus and neighboring parahippocampal and rhinal cortex structures (Cohen et al., 1997; Etchamendy et al., 2001; Squire, 1998).

Etchamendy et al. (2001) used this two-stage paradigm to evaluate a possible association between the cognitive impairments in aged mice and the down regulation of retinoid signaling (Etchamendy et al., 2001). Implicit memory (Stage 1) was unimpaired in aged mice, but an explicit memory deficit (Stage 2) was completely alleviated by administration of retinoic acid. Hippocampal levels of retinoid receptors, expression of specific target genes associated with these receptors, and hippocampal LTP were also restored to near-young adult levels after acute administration of retinoic acid. All of these facilitative effects of RA could be abolished by the co-administration of a retinoic acid receptor antagonist. These findings suggest that retinoic acid can alter hippocampal-dependent processes. In another test using this same paradigm, Marighetto et al. (2000) examined hippocampectomized rats and found that performance was impaired only in tasks that emphasized comparison of items (Stage 2) and not those that encouraged separate representations for individual items (Stage 1). This finding further supports the theory that explicit memory requires relational representations of past experiences and that these comparisons are hippocampal-dependent (Marighetto et al., 2000).

Utilizing this same paradigm, a major goal of the present study was to investigate whether adult exposure to ISO alters implicit or explicit learning and memory processes in the rat. Previous ISO studies have focused on two aspects of behavior: depression-like behaviors and learning/memory effects. Despite evidence (Crandall et al., 2004; Sakai et al., 2004) that 13-*cis*-RA suppresses hippocampal cell proliferation, neurogenesis, and survival (similar to findings in depressed patients), Ferguson et al. (2005) found that chronic 13-*cis*-RA exposure did not severely affect depression-like behaviors in rats. Three studies have specifically looked at learning and memory effects of 13-*cis*-RA in adults, but have found apparently discrepant results. Crandall et al. (2004) found that 13-*cis*-RA exposure reduced hippocampal neurogenesis and performance on the hippocampal-dependent radial-arm maze task. However, Ferguson and Berry (2007) found no evidence of learning and memory effects of 13-*cis*-RA, despite testing rats in three different spatial tasks (Morris water maze, 8-arm radial maze, and a dry land maze) which each used a different type of reinforcer (i.e., water escape, food reinforcement, or water reinforcement). In a third study, O'Donnell et al. (2003) found that, similar to Etchamendy et al.'s (2001) findings with aged mice, 13-*cis*-RA reversed the amnesic effects of dimethyl sulphoxide (DMSO) exposure using conditioned avoidance and Morris water maze tasks.

In the current study, animals were chronically exposed to ISO and subjected to four behavioral tests intended to more fully evaluate the effects of ISO on learning and memory in adult rats and rule out non-specific effects of the drug (e.g., motor activity or anxiety effects). First, animals were tested in both stages of the two-stage implicit/explicit memory task. Animals were then tested in an open field task, which is widely used to examine anxiety levels in animals as demonstrated by their activity levels and overall interaction with the environment (i.e., motor activity) (Goto et al., 1993). Third, animals were tested in an elevated plus maze. The elevated plus maze is commonly used to study anxiety (Hogg, 1996) based on the natural aversion of rodents to explore open spaces as well as the innate fear rodents have for