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Anxiogenic-like effects of chronic nicotine exposure in zebrafish

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ABSTRACT

Nicotine is one of the most widely used and abused legal drugs. Although its pharmacological profile has been extensively investigated in humans and rodents, nicotine CNS action remains poorly understood. The importance of finding evolutionarily conserved signaling pathways, and the need to apply high-throughput in vivo screens for CNS drug discovery, necessitate novel efficient experimental models for nicotine research. Zebrafish (*Danio rerio*) are rapidly emerging as an excellent organism for studying drug abuse, neuropharmacology and toxicology and have recently been applied to testing nicotine. Anxiolytic, rewarding and memory-modulating effects of acute nicotine treatment in zebrafish are consistently reported in the literature. However, while nicotine abuse is more relevant to long-term exposure models, little is known about chronic effects of nicotine on zebrafish behavior. In the present study, chronic 4-day exposure to 1–2 mg/L nicotine mildly increased adult zebrafish shoaling but did not alter baseline cortisol levels. We also found that chronic exposure to nicotine evokes robust anxiogenic behavioral responses in zebrafish tested in the novel tank test paradigm. Generally paralleling clinical and rodent data on anxiogenic effects of chronic nicotine, our study supports the developing utility of zebrafish for nicotine research.

1. Introduction

Like alcohol and caffeine, nicotine (Fig. 1) is one of the most widely used legal drugs (Crocq, 2003). Acting as a classical agonist of nicotinic acetylcholine receptors, it modulates the release of several neurotransmitters, including dopamine, norepinephrine and serotonin (Dong et al., 2010; Eddins et al., 2009, 2010), also see (Clarke and Reuben, 1996; Ribeiro et al., 1993; Wonnacott, 1997) for details. Used as a mild psychostimulant by healthy individuals, nicotine is also a major substance of abuse (NIDA, 2014; WHO, 2013), causing strong dependence and harm to the users (Fig. 1AB; Gable, 2006; Morgan et al., 2010; Nutt et al., 2010). Not surprisingly, nicotine is ranked among the top 5 most dangerous abused drugs, after alcohol, heroin, cocaine and methamphetamine (Carhart-Harris and Nutt, 2013; Nutt et al., 2010).

Nicotine use is currently the main preventable cause of disease, disability and death worldwide (CDC, 2014; NIDA, 2014). While nicotine abuse occurs due to tobacco intake, tobacco contains a variety of other toxic chemicals that account for much of the harm, in addition to that caused by nicotine (Nutt et al., 2014; Rose et al., 2010). Nicotine abuse is widespread, currently affecting ~30% of the global adult population and in some countries exceeding 50% (Bank, 2014; WHO, 2013); Fig. 1C. Nicotine abuse is particularly increased among drug addicts, operating as a 'gateway drug' to increase brain susceptibility to other drugs of abuse, such as cocaine (Levine et al., 2011). Nicotine use is also highly comorbid with other psychiatric disorders, with bidirectional influences observed between the disorder and nicotine dependence (Griesler et al., 2011). Collectively, this implicates nicotine use as a major biomedical and societal problem, meriting further in-depth translational studies.

Acute nicotine exposure evokes positive reinforcing effects, including mild euphoria, heightened arousal, diminished appetite, and reduced anxiety, while also enhancing aspects of attention and cognition, in human subjects (Heishman et al., 2010; Pomerleau and Pomerleau, 1992; Pomerleau et al., 1984). However, whereas chronic nicotine users report an overall profile of rewarding sensations, increased comfort and reduced negative mood, such states are accompanied by negative effects, such as tension and jitteriness (Cohen and George, 2013; Henningfield and Goldberg, 1983; Rose et al., 2010; Sofuoglu et al., 2008). Moreover, the perceived calming effects of continued nicotine exposure may simply represent the reversal of the negative affect induced by nicotine deprivation, thereby forming an overall anxiogenic profile (Cohen and George, 2013; Parrott, 1995, 1998).

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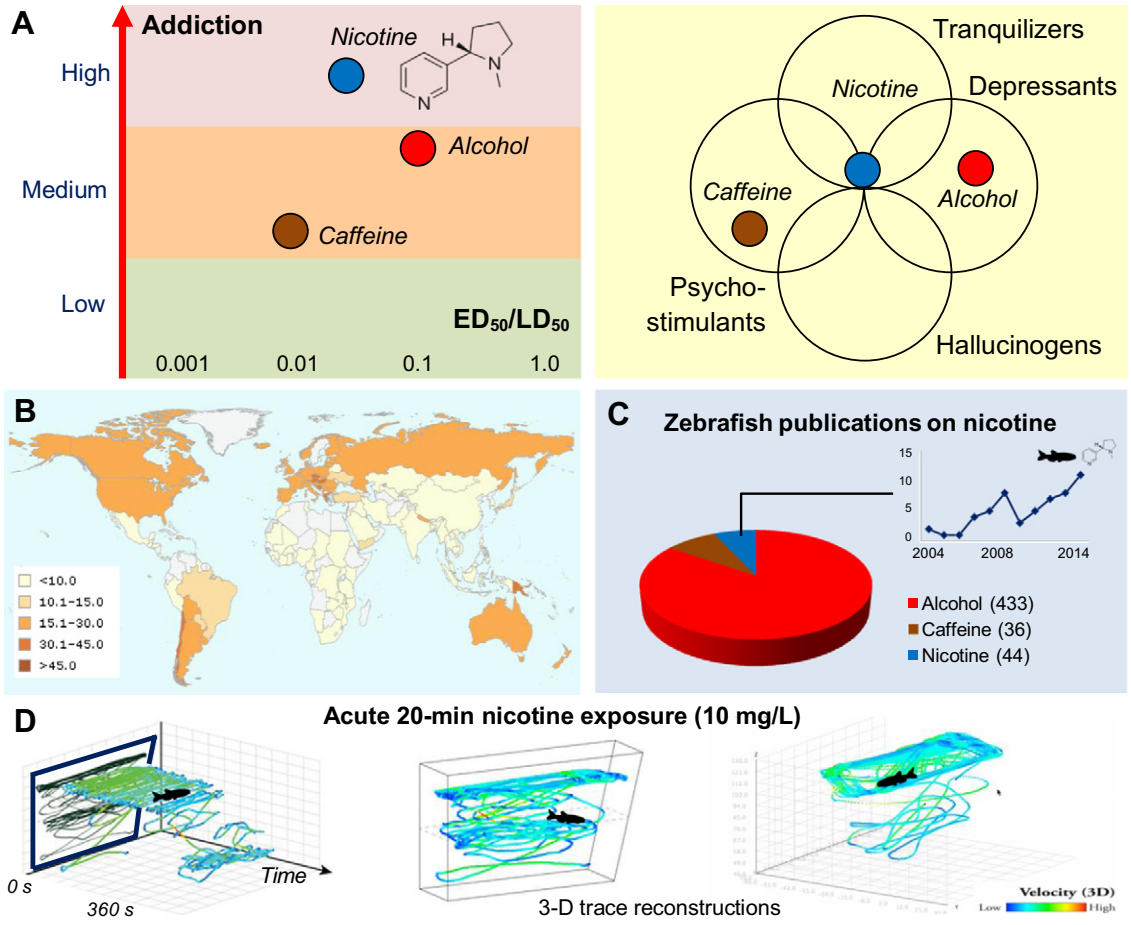

Fig. 1. The growing utility of zebrafish models for nicotine research. (A) Summary of addictive potential and toxic properties (ED_{50}/LD_{50} ratio) of acute nicotine, compared to alcohol and caffeine, according to (Gable, 2006). Right subpanel: classification of main classes of psychotropic drugs. (B) Global tobacco smoking statistics (% of the population) among adults (WHO, 2013), based on graphs freely available in the public domain via Wikipedia. (C) The growing body of zebrafish literature on nicotine, based on PubMed search (December 2014). (D) examples of anxiolytic-like behavior typically produced by acute nicotine (10 mg/L) in the novel tank test (modified from (Cachat et al., 2011), published under the Creative Commons Attribution license).

Complementing clinical evidence, nicotine's psychopharmacological profile has been extensively investigated in various animal models (Matta et al., 2007). For example, acute administration of nicotine at various doses in rodent models evokes robust anxiolytic-like responses (Elliott et al., 2004; Irvine et al., 1999); also see similar effects produced by other cholinergic agonists (Brioni et al., 1994; Turner et al., 2010). Paralleling clinical findings, chronic administration induces consistent anxiogenic-like behavior in rodents treated with nicotine (Caldarone et al., 2008; Elliott et al., 2004; Mesa-Gresa et al., 2013) and some other nicotinic agonists (Turner et al., 2010) (see, however, (Turner et al., 2011)).

Several considerations necessitate novel approaches to studying nicotine action, as our increased understanding of its molecular effects and genetic contributions may lead to improved treatment strategies (Klee et al., 2011, 2012). For example, nicotine research and screening of drugs with anti-nicotine properties benefit markedly from the availability of high-throughput, low-cost alternatives to rodent models.

Being a vertebrate species with high genetic and physiological homology to mammals, zebrafish (*Danio rerio*) are rapidly emerging as an excellent model organism for studying pharmacology and toxicology of various CNS drugs (Grossman et al., 2010; Maximino et al., 2014; Neelkantan et al., 2013; Tran et al., 2014; Wong et al., 2010b), including nicotine (Cousin et al., 2014; Eddins et al., 2010; Klee et al., 2011; Levin, 2011). Moreover, zebrafish have a sophisticated molecular genetic tool set, markedly increasing their value and application for pharmacogenetic research. For example, in addition to *N*-ethyl-*N*-nitrosourea (ENU)-induced (Mullins et al., 1994) and viral vector-mediated (Amsterdam et al., 1999) mutagenesis or morpholino-evoked gene silencing, other genetic tools in zebrafish include 'gene-breaking transposon' screens (Petzold et al., 2009), 'clustered regularly interspaced short palindromic repeats' (CRISPR) (Hwang et al., 2013), 'transcription activator-like effector nuclease' (TALEN) (Zu et al., 2013) and 'targeting induced local lesions in genomes' (TILLING) (Moens et al., 2008). Several genetically modified zebrafish strains, developed using such methods and possessing altered nicotine responses, have already been reported (Petzold, 2010; Petzold et al., 2009). Finally, the importance of identifying evolutionarily conserved mechanisms of drug actions further necessitate expanding the range of model organisms, recognized as a key strategic direction for drug discovery (Cousin et al., 2014; Klee et al., 2012; Robinson et al., 2013; Stewart et al., 2014).

Behavioral effects of acute nicotine treatment in zebrafish, previously reported in the literature (Klee et al., 2011), include anxiolytic-like action and potent modulation of cognitive responses in various memory/learning tasks (Bencan and Levin, 2008; Eddins et al., 2009; Levin, 2011; Levin et al., 2006, 2007; Levin and Chen, 2004). In addition, nicotine evokes psychostimulant (Petzold, 2010; Petzold et al., 2009) and robust reward-like (e.g., preference) behavior in zebrafish (Cousin et al., 2014; Kedikian et al., 2013; Kily et al., 2008; Klee et al., 2012), making these fish an excellent model organism to study nicotine