and behavioral responses to olfactory social cues, and high-repetitive self-grooming. BTBR were given opportunities to interact with C57BL/6J (B6), an inbred strain with high-sociability and low-repetitive behaviors.

First, we attempted to model behavioral interventions given by caretakers to young autistic children. Newborn BTBR were cross-fostered with B6 mothers. Cross-fostering produced no significant effects on social or repetitive behaviors in B6 and BTBR mice, either at juvenile or adult ages. B6 and BTBR raised by dams of the opposite strain showed behaviors similar to those raised by foster dams of the same strain and those raised by their biological mothers (Yang *et al*, 2007). This finding is consistent with the rejection of the early 'refrigerator mother' explanation of autism.

Second, we modeled peer interventions in older children and adolescents with autism (Reichow and Volkmar, 2010). Juvenile BTBR were reared with juvenile B6, beginning at weaning. BTBR who lived with B6 cagemates during juvenile ages developed high sociability as adults, whereas control BTBR who lived with BTBR cagemates continued to show social deficits (Yang *et al*, 2011).

Third, we are now engaged in understanding the specific behaviors occurring between BTBR and B6 juveniles in their shared home cages, which might lead to improved sociability in BTBR adults. Video recordings of home cages during the dark phase, when mice are awake and interactive, are being scored on measures including social investigation, proximity states, aggressive interactions, and activity levels. Preliminary observations suggest that BTBR housed with B6 cagemates receive more social investigation than BTBR housed with BTBR cagemates. It is possible that increased exposure to social solicitation behaviors as juveniles may be facilitating the adult sociability seen in BTBR reared with B6 cagemates.

Animal models of autism will need to meet the standard criteria of face validity (analogous symptoms, such as social deficits), construct validity (analogous causes, such as genetic mutations), and predictive validity (analogous responses to treatments). Evidence that an early behavioral intervention rescued adult sociability in BTBR mice gives credence to the predictive value of this mouse model.

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Phospholipase D as a Therapeutic Target in Brain Disorders

Phospholipid-mediated signaling pathways control a myriad of physiological processes including various aspects of brain function. Among the phospholipid enzyme families, phospholipase D (PLD) is emerging as a key player in regulating phospholipid metabolism, and a newly appreciated therapeutic target for Alzheimer's disease (AD), stroke, and other brain disorders (Oliveira and Di Paolo, 2010). PLD catalyzes the conversion of phosphatidylcholine to the lipid second messenger phosphatidic acid and choline. Two mammalian isoforms of conventional PLDs have been identified, PLD1 and PLD2, which share 53% sequence identity and are subject to different regulatory mechanisms. Previous research relied on the overexpression of either catalytically active or inactive forms of either PLD1 or PLD2 in cells, or employed siRNA for the individual isoforms in an effort to discern discrete roles for PLD1 and PLD2 in brain disorders (Oliveira and Di Paolo, 2010). In 2010, $PLD1^{-/-}$ and $PLD2^{-/-}$ mice developed via gene targeting were reported, clearly defining, nonoverlapping roles, and therapeutic potential for both PLD1 and PLD2 in the pathogenesis of AD. From overexpression and biochemical studies, it has been shown that PLD1 (but not PLD2) regulates the trafficking of APP and the assembly of the γ-secretase complex via a direct interaction with PS1 (Cai *et al*, 2006). In 2010, $PLD2^{-/-}$ mice provided the first *in vivo* evidence implicating PLD in AD. Here, PLD2 was shown to be required for the synaptotoxic action of Aβ, and that PLD2 ablation rescues memory deficits and engenders synaptic protection in SwAPP mice, despite a high Aβ load (Oliveira *et al*, 2010). Also in 2010, $PLD1^{-/-}$ mice were shown to display impaired $\alpha_{IIb}\beta_3$ intergrin activation and defective glycoprotein 1b-dependent aggregate formation, leading to protection from thrombosis and ischemic brain injury without increasing bleeding time (Elvers *et al*, 2010). Historically, few small molecule tools existed to study PLD function, and none of the inhibitors displayed PLD isoform-selective inhibition. The classical biochemical approach relies on

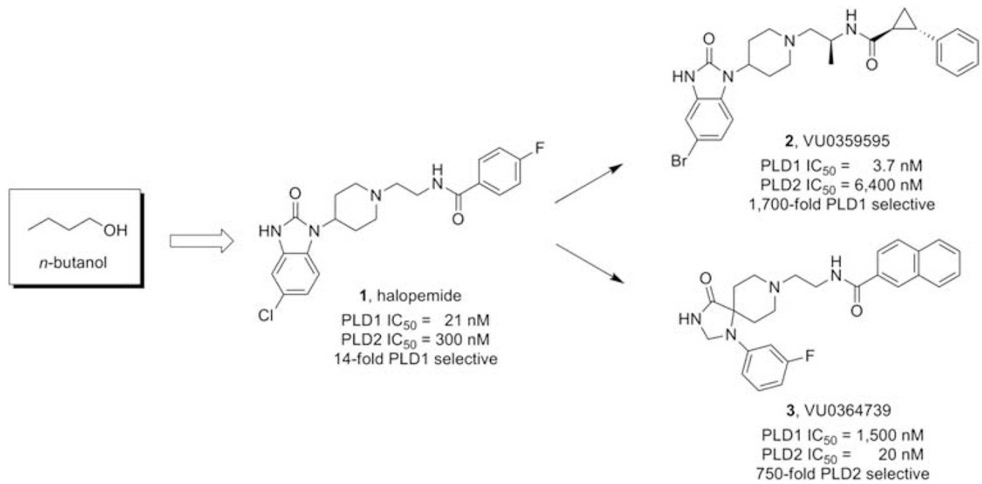

Figure 1. Evolution of small molecule, isoform-selective PLD inhibitors.

n-butanol, a small molecule that is not a PLD inhibitor, rather *n*-butanol blocks PLD-catalyzed phosphatidic acid production by competing with water as a nucleophile, thereby generating phosphatidylbutanol in a competitive transphosphatidylation reaction. A renaissance in the PLD inhibitor field began in 2007 when halopemide (**1**), a psychotropic agent discovered in the late 1970s, was shown to be a dual PLD1/2 inhibitor (Scott *et al*, 2009). More importantly, **1** has been in humans in five clinical trials and was shown to be safe and effective; thus inhibition of PLD with a small molecule is a viable therapeutic approach, a finding also noted in the PLD KO mice. Using **1** as a lead, a diversity-oriented synthesis campaign was pursued by the Brown and Lindsley labs, where ~1000 analogs of **1** were synthesized and evaluated in cell-based and biochemical PLD1 and PLD2 assays (Scott *et al*, 2009). From this effort, isoform-selective PLD1 (**2**) and PLD2 (**3**) inhibitors were developed with low nanomolar potencies, unprecedented PLD isoform selectivity and DMPK profiles to allow *in vivo* target validation studies to be pursued (Lavieri *et al*, 2010; Figure 1).

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Transferrin Antibodies into the Brain

Opening the central nervous system (CNS) to antibody therapies would substantially improve our ability to selectively target neurological disease. However, brain uptake of antibodies is limited by the presence of the blood–brain barrier (BBB). Over the past 20 years, progress has been made in designing methods to improve uptake of antibodies via molecular engineering, with most attention being placed on utilizing the BBB's endogenous mechanisms to transport proteins into the brain, known as receptor-mediated transcytosis (RMT; Jones and Shusta, 2007). Nevertheless, challenges in both understanding the biology of BBB transport and in engineering antibodies to optimally cross the BBB remain. In particular, the majority of studies assessing RMT pathways at the BBB have relied on radiolabeled proteins. However, from a drug development standpoint, success is only achieved if antibody is delivered to the brain in sufficient