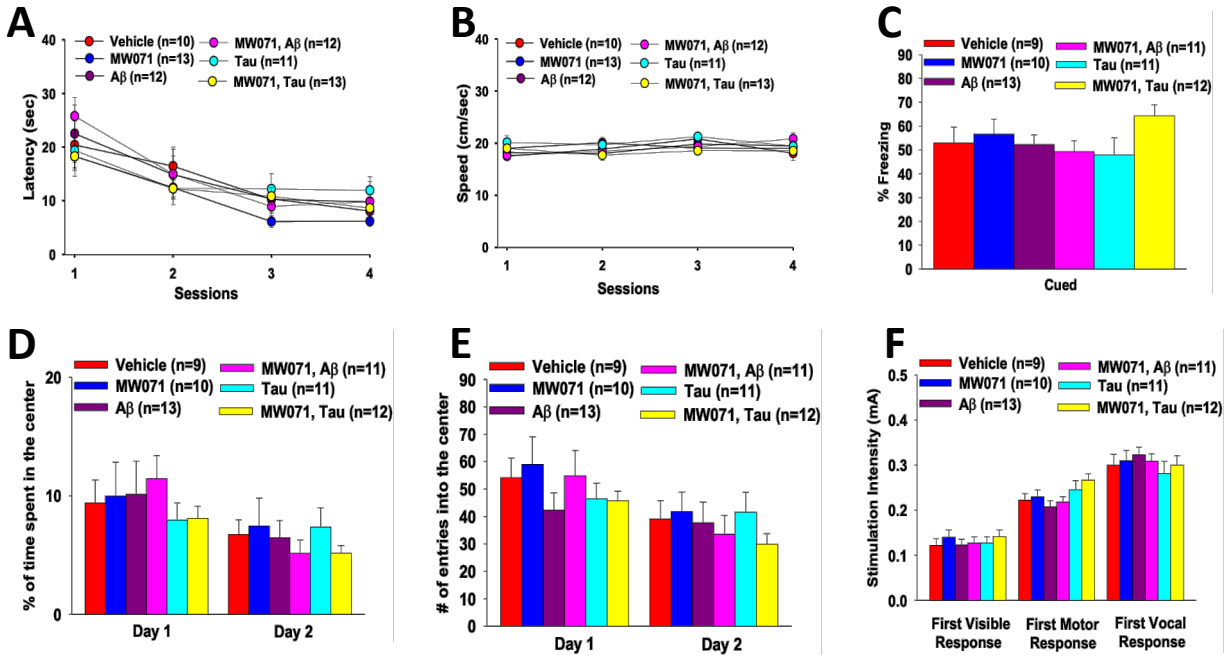
Supplementary Material

The 5HT2b Receptor in Alzheimer's Disease: Increased Levels in Patient Brains and Antagonist Attenuation of Amyloid and Tau Induced Dysfunction



Supplementary Figure 1. Control behavioral analyses for MW071 suppression of memory deficits by Aβ- or Tau-oligomers. A, B) Testing with the visible platform task does not reveal any difference in (A) time to reach the visible platform (ANOVA for repeated measures: $F(5,65)=0.4625$, $p=0.8027$) and (B) average speed (ANOVA for repeated measures: $F(5,65)=0.6686$, $p=0.6487$). Vehicle: N=10 (5 males, 5 females), MW071: N=13 (6 males, 7 females), Aβ: N=12 (6 males, 6 females), Aβ+ MW071: N=12 (6 males, 6 females), Tau: N=11 (5 males, 6 females), Tau+ MW071: N=13 (7 males, 6 females). C) Freezing responses during the auditory cued conditioning were the same among the groups (one-way ANOVA: $F(5,60)=1.203$, $p=0.3189$). Vehicle: N=9 (5 males, 4 females), MW071: N=10 (5 males, 5 females), Aβ: N=13 (7 males, 6 females), Aβ+MW071: N=11 (6 males, 5 females), Tau: N=11 (5 males, 6 females), Tau+MW071: N=12 (6 males, 6 females). D, E) Open field test showed a similar percentage of time spent in the center (D) (one-way ANOVA: day 1: $F(5,60)=0.3921$, $p=0.8524$; day 2: $F(5,60)=0.4895$, $p=0.7828$) and (E) number of entries into the center among all conditions (one-way ANOVA: day 1: $F(5,60)=0.8351$, $p=0.53$; day 2: $F(5,60)=0.5069$, $p=0.7699$), indicating no differences in exploratory behavior. Vehicle: N=9 (5 males, 4 females), MW071: N=10 (5 males, 5 females), Aβ: N=13 (7 males, 6 females), Aβ+MW071: N=11 (6 males, 5 females), Tau: N=11 (5 males, 6 females), Tau+MW071: N=12 (6 males, 6 females). F) No difference was detected among the groups during assessment of the sensory threshold. One-way ANOVA among all: for visible response $F(5,60)=0.3507$, $p=0.8798$; for motor response $F(5,60)=2.083$, $p=0.0800$ and for audible response $F(5, 60)=0.4479$, $p=0.8132$. Vehicle: N=9 (5 males, 4 females), MW071: N=10 (5 males, 5 females), Aβ: N=13 (7 males, 6 females), Aβ+MW071: N=11 (6 males, 5 females), Tau: N=11 (5 males, 6 females), Tau+MW071: N=12 (6 males, 6 females).

Supplementary Table 1. List of human frozen brain used for the 5HT2bR western blot quantification.

Subject #	diagnosis	age	gender	PMI	Braak #	CERAD #
1	HC	82	W	3:50	III	0
2	AD	86	W	5:10	VI	C
3	AD	89	W	3:45	VI	C
4	AD	89+	W	3:20	VI	C
5	AD	89+	W	3:10	V	B
6	HC	89+	W	2:55	III	0
7	HC	89	M	4:47	III	0
8	AD	88	W	14:02	VI	C
9	AD	81	W	6:30	VI	C
10	HC	89+	W	2:00	IV	A
11	AD	89+	M	29:30	VI	C
12	HC	79	W	2:57	III	NA
13	AD	71	M	NaN	VI	C
14	AD	89	M	NaN	VI	C
15	AD	89	W	2:40	VI	C
16	AD	89+	W	5:30	V	B
17	AD	89	M	4:04	V	B
18	AD	84	M	2:00	IV	C
19	AD	79	M	NaN	VI	C
20	HC	80	M	NaN	0	A
21	AD	81	M	9:40	V	B
22	AD	80	W	4:20	IV	B
23	HC	89+	M	4:47	III	A
24	AD	89+	W	3:10	VI	C
25	HC	89+	W	2:55	III	NA
26	AD	89+	W	22:55	VI	C
27	HC	89+	M	3:00	IV	A
28	AD	89+	M	2:24	V	B
29	AD	86	M	3:00	V	B
30	AD	88	W	0:05	V	C
31	HC	62	M	4:00	0	NA
32	AD	89	W	2:40	VI	C
33	AD	89+	W	11:00	III	A
34	HC	62	M	NaN	I	NA
35	AD	89+	W	1:25	IV	B
36	AD	89+	M	NaN	IV	C
37	AD	89+	W	NaN	IV	B
38	AD	79	M	NaN	IV	B
39	AD	77	M	6:43	VI	C
40	AD	75	W	23:35	VI	C
41	AD	68	M	13:30	VI	C
42	HC	74	M	10:53	III	A

43	AD	88	W	6:45	VI	C
44	AD	74	W	23:09	VI	C
45	HC	56	M	8:50	II	NA
46	AD	75	M	6:10	VI	C
47	HC	60	M	7:07	II	0
48	AD	79	M	23:45	VI	C
49	AD	89	W	12:40	VI	B
50	AD	88	M	16:55	VI	C
51	AD	89+	W	14:35	VI	C
52	AD	75	W	23:35	VI	C
53	AD	94	W	9:45	VI	C
54	AD	86	M	12:50	V	B
55	HC	74	M	18:46	III	0
56	HC	80	M	31:55	II	A
57	HC	85	M	14:55	IV	A
58	HC	84	W	21:15	II	NA
59	HC	75	M	36:17	III	NA

In the table, for every sample is specified the health condition, age, gender, and Braak and CERAD index. The average age at the time of death for AD patients is 82.87 ± 2.15, whereas the average death age for controls is 78.53 ± 2.59. (All individuals that were 89+ were computed as 90 years old for the average). PMI, Postmortem Interval calculated from the patients reported time of death to the time the patient was brought into the cold room. NaN, not a number, i.e., the calculation failed, because the time of death or the time when the patient was brought to the cold room was not reported. NA, not available.

Supplementary Table 2. MW071 Lacks Off-Target Kinase Inhibitor Activity in Kinome-Wide Screen

Abl(h)	CK1δ(h)	Flt3(D835Y)(h)	Mer(h)	PKBα(h)	SRPK2(h)
Abl(m)	CK1(y)	Flt3(h)	Met(h)	PKBβ(h)	STK25(h)
Abl (H396P) (h)	CK2(h)	Flt4(h)	Met(D1246H)(h)	PKBγ(h)	STK33(h)
Abl (M351T)(h)	CK2α2(h)	Fms(h)	Met(D1246N)(h)	PKCα(h)	Syk(h)
Abl (Q252H) (h)	CLK1(h)	Fms(Y969C)(h)	Met(M1268T)(h)	PKCβI(h)	TAK1(h)
Abl(T315I)(h)	CLK2(h)	Fyn(h)	Met(Y1248C)(h)	PKCβII(h)	TAO1(h)
Abl(Y253F)(h)	CLK3(h)	GCK(h)	Met(Y1248D)(h)	PKCγ(h)	TAO2(h)
ACK1(h)	CLK4(h)	GRK5(h)	Met(Y1248H)(h)	PKCδ(h)	TAO3(h)
ALK(h)	cKit(h)	GRK6(h)	MINK(h)	PKCε(h)	TBK1(h)
ALK4(h)	cKit(D816V)(h)	GRK7(h)	MKK4(m)	PKCη(h)	Tec(h) activated
Arg(h)	cKit(D816H)(h)	GSK3α(h)	MKK6(h)	PKCι(h)	TGFBR1(h)
AMPKα1(h)	cKit(V560G)(h)	GSK3β(h)	MKK7β(h)	PKCμ(h)	Tie2 (h)
AMPKα2(h)	cKit(V654A)(h)	Haspin(h)	MLCK(h)	PKCθ(h)	Tie2(R849W)(h)
Arg(m)	CSK(h)	Hck(h)	MLK1(h)	PKCζ(h)	Tie2(Y897S)(h)
ARK5(h)	c-RAF(h)	Hck(h) activated	Mnk2(h)	PKD2(h)	TLK2(h)
ASK1(h)	cSRC(h)	HIPK1(h)	MRCKα(h)	PKG1α(h)	TrkA(h)
Aurora-A(h)	DAPK1(h)	HIPK2(h)	MRCKβ(h)	PKG1β(h)	TrkB(h)
Aurora-B(h)	DAPK2(h)	HIPK3(h)	MSK1(h)	Plk1(h)	TrkC(h)
Aurora-C(h)	DCAMKL2(h)	IGF-1R(h)	MSK2(h)	Plk3(h)	TSSK1(h)
Axl(h)	DDR2(h)	IGF-1R(h), activated	MSSK1(h)	PRAK(h)	TSSK2(h)
Blk(h)	DMPK(h)	IKKα(h)	MST1(h)	PRK2(h)	Txk(h)
Blk(m)	DRAK1(h)	IKKβ(h)	MST2(h)	PrKX(h)	TYK2(h)
Bmx(h)	DYRK2(h)	IKKε(h)	MST3(h)	PTK5(h)	ULK2(h)
BRK(h)	eEF-2K(h)	IR(h)	MST4(h)	Pyk2(h)	ULK3(h)
BrSK1(h)	EGFR(h)	IR(h), activated	mTOR(h)	Ret(h)	Wee1(h)
BrSK2(h)	EGFR(L858R)(h)	IRR(h)	mTOR/FKBP12(h)	Ret (V804L)(h)	WNK2(h)
BTK(h)	EGFR(L861Q)(h)	IRAK1(h)	MuSK(h)	Ret(V804M)(h)	WNK3(h)
BTK(R28H)(h)	EGFR(T790M)(h)	IRAK4(h)	NEK2(h)	RIPK2(h)	VRK2(h)
B-Raf(h)	EGFR(T790M,L858R)(h)	Itk(h)	NEK3(h)	ROCK-I(h)	Yes(h)
B-Raf(V599E)(h)	EphA1(h)	JAK1(h)	NEK6(h)	ROCK-II(h)	ZAP-70(h)
CaMKI(h)	EphA2(h)	JAK2(h)	NEK7(h)	ROCK-II(r)	ZIPK(h)
CaMKIIβ(h)	EphA3(h)	JAK3(h)	NEK11(h)	Ron(h)	PI3 Kinase (p110b/p85a)(h)
CaMKIIγ(h)	EphA4(h)	JNK1α1(h)	NLK(h)	Ros(h)	PI3 Kinase (p120g)(h)
CaMKIδ(h)	EphA5(h)	JNK2α2(h)	p70S6K(h)	Rse(h)	PI3 Kinase (p110d/p85a)(h)
CaMKIIδ(h)	EphA7(h)	JNK3(h)	PAK1(h)	Rsk1(h)	PI3 Kinase (p110a/p85a)(m)
CaMKIV(h)	EphA8(h)	KDR(h)	PAK2(h)	Rsk1(r)	PI3 Kinase (p110a/p65a)(m)
CDK1/cyclinB(h)	EphB2(h)	Lck(h)	PAK4(h)	Rsk2(h)	PI3 Kinase (p110a(E545K)/p85a)(m)
CDK2/cyclinA(h)	EphB1(h)	Lck(h) activated	PAK5(h)	Rsk3(h)	PI3 Kinase (p110a(H1047R)/p85a)(m)
CDK2/cyclinE(h)	EphB3(h)	LIMK1(h)	PAK6(h)	Rsk4(h)	PI3 Kinase (p110b/p85b)(m)
CDK3/cyclinE(h)	EphB4(h)	LKB1(h)	PAR-1Bα(h)	SAPK2a(h)	PI3 Kinase (p110b/p85a)(m)
CDK5/p25(h)	ErbB4(h)	LOK(h)	PASK(h)	SAPK2a(T106M)(h)	PI3 Kinase (p110d/p85a)(m)
CDK5/p35(h)	FAK(h)	Lyn(h)	PEK(h)	SAPK2b(h)	PI3 Kinase (p110a(E542K)/p85a)(m)
CDK6/cyclinD3(h)	Fer(h)	Lyn(m)	PDGFRα(h)	SAPK3(h)	PI3 Kinase (p110a/p85a)(h)
CDK7/cyclinH/MAT1(h)	Fes(h)	LRRK2(h)	PDGFRα(D842V)(h)	SAPK4(h)	PI3 Kinase (p110a(E542K)/p85a)(h)
CDK9/cyclin T1(h)	FGFR1(h)	MAPK1(h)	PDGFRα(V561D)(h)	SGK(h)	PI3 Kinase (p110a(H1047R)/p85a)(h)
CHK1(h)	FGFR1(V561M)(h)	MAPK2(h)	PDGFRβ(h)	SGK2(h)	PI3 Kinase (p110a(E545K)/p85a)(h)
CHK2(h)	FGFR2(h)	MAPK2(m)	PDK1(h)	SGK3(h)	PI3 Kinase (p110a/p65a)(h)
CHK2(I157T)(h)	FGFR2(N549H)(h)	MAPKAP-K2(h)	PhKγ2(h)	SIK(h)	PI3KC2a(h)
CHK2(R145W)(h)	FGFR3(h)	MAPKAP-K3(h)	Pim-1(h)	Snk(h)	PI3KC2g(h)
CK1γ1(h)	FGFR4(h)	MEK1(h)	Pim-2(h)	Src(1-530)(h)	PIP4K2a(h)
CK1γ2(h)	Fgr(h)	MARK1(h)	Pim-3(h)	Src(T341M)(h)	PIP5K1a(h)
CK1γ3(h)	Flt1(h)	MELK(h)	PKA(h)	SRPK1(h)	PIP5K1g(h)