SUPPLEMENTAL DATA

Title: Positive allosteric modulation of the muscarinic M_1 receptor improves efficacy of antipsychotics in mouse glutamatergic deficit models of behavior

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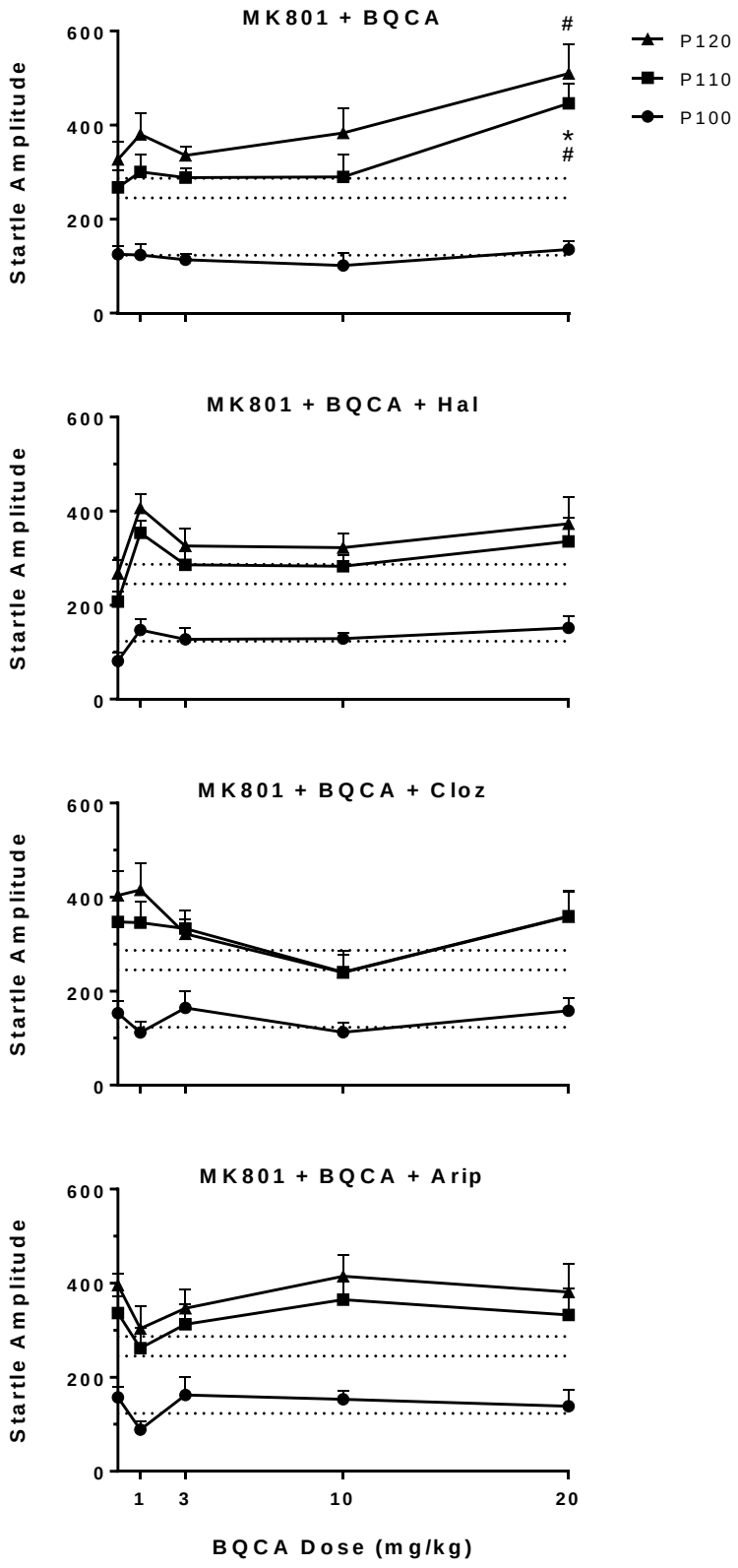

MK801 + BQCA
MK801 + BQCA + Hal
MK801 + BQCA + Cloz
MK801 + BQCA + Arip
Startle Amplitude
600
400
200
0
#
*
#
P120
P110
P100
1
3
10
20
BQCA Dose (mg/kg)

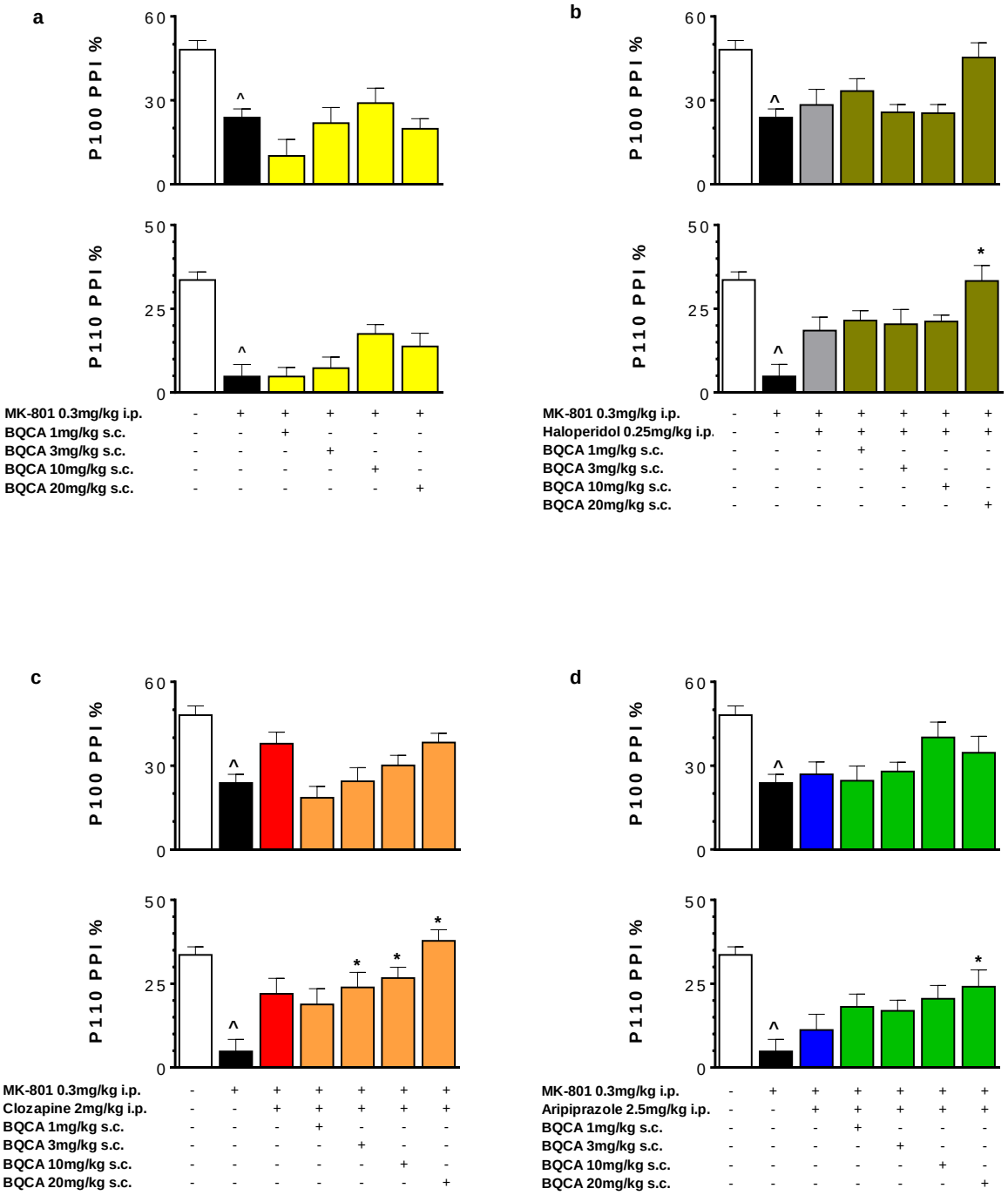
1

2

3

Supplemental Fig.1

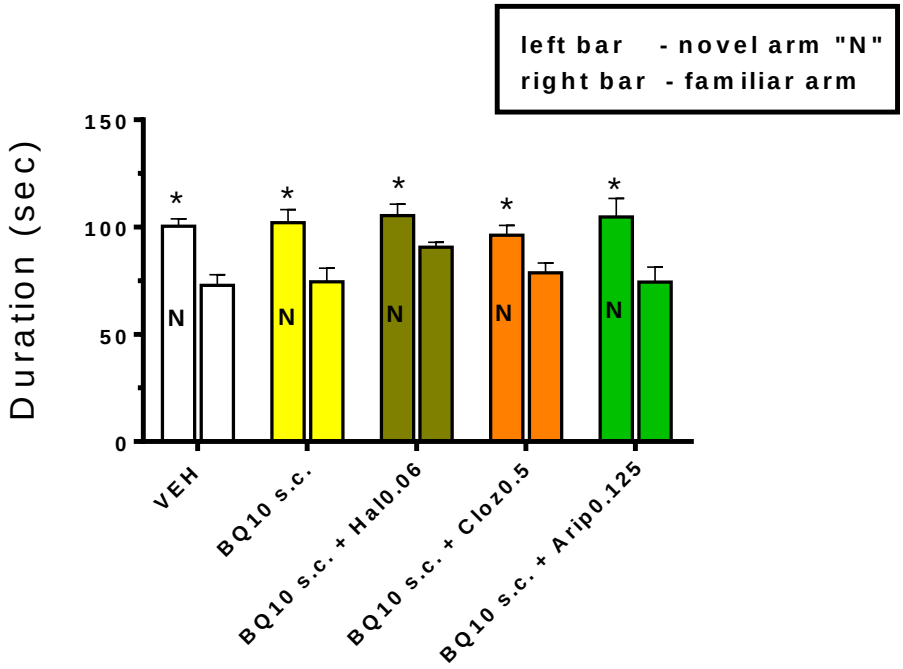
Startle amplitude measured at pulse alone (p100, 110 and 120) during the PPI test remained unaffected by drug pretreatment, except mice that were treated by 20 mg/kg (s.c.) of BQCA and MK-801 (0.3 mg/kg). In particular, the higher startle amplitude is observed in tested mice measured at 110 and 120db stimulus (top panel).
Data are mean ± SEM, * $p < 0.05$ vs MK-801 control, and $^{\#}$ $p < 0.05$ vs vehicle control (-----).



Supplemental Fig. 2.

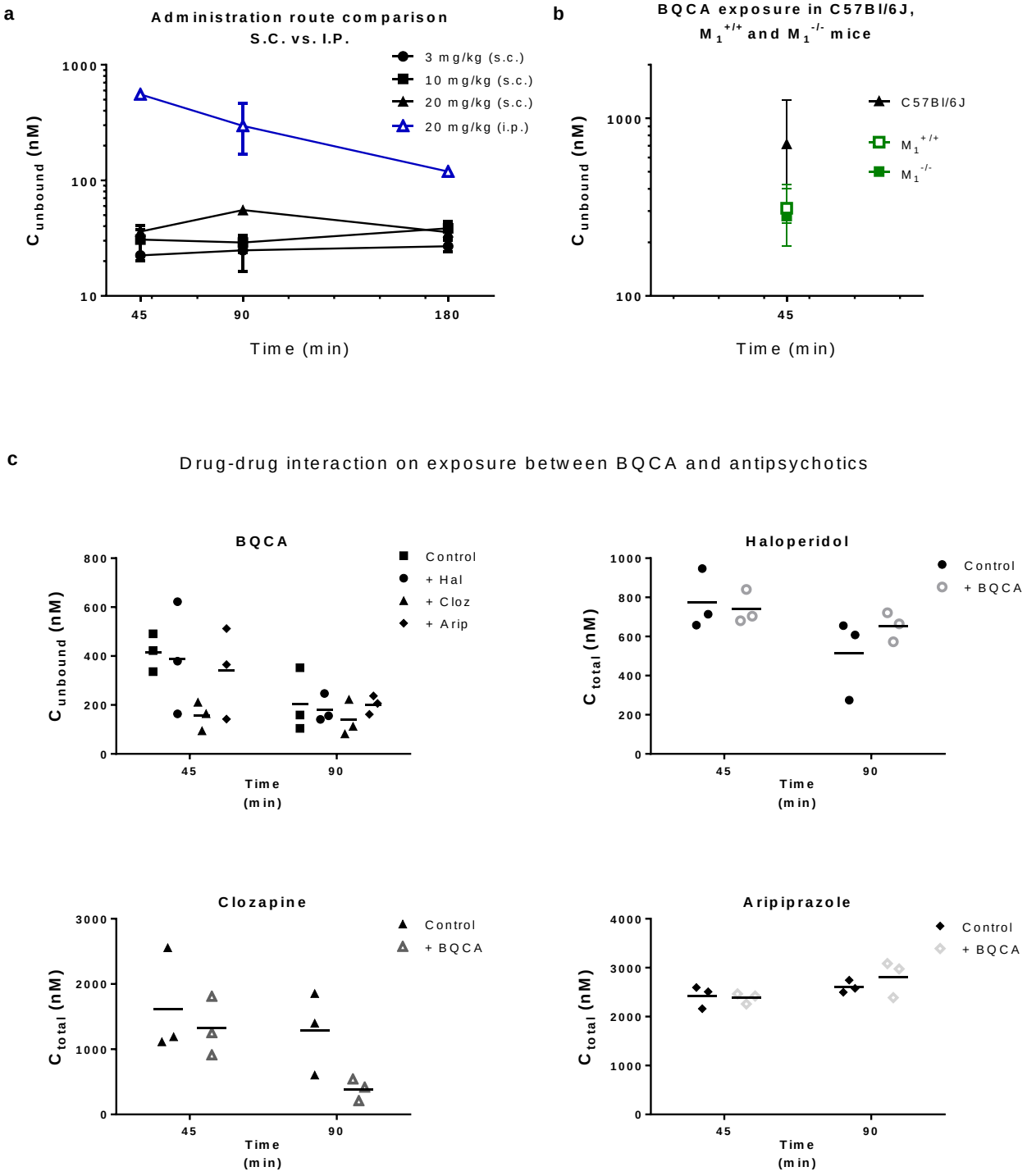
PPI measured at p100 and p110 exhibited a similar effect as observed at p120. As mentioned in Materials and Methods, mice were tested in PPI of startle elicited by acoustic stimulus of 100, 110 and 120db, and prepulses. PPI measured at 120db is presented in the main text. PPI measured at p100 and p110 either shared a similar reversal seen in p120 or a trend of reversal, by combination of BQCA and antipsychotics.

Data are mean ± SEM, $^{\wedge}p < 0.05$ for difference between vehicle vs MK-801 controls, as indication of PPI disruption by MK-801; * $p < 0.05$ for difference between groups vs. MK-801 control for a significant reversal of PPI disruption.



Supplemental Fig. 3.

Memory function was not affected by pretreatment of BQCA alone, or combined BQCA and antipsychotics treatment, in mice without MK-801 treatment, whereas C57Bl/6J mice received BQCA (10 mg/kg s.c.) and clozapine (1 mg/kg), or aripiprazole (0.25 mg/kg) exhibited insufficient exploratory activity, and therefore excluded.
* $p < 0.05$ for difference between novel and familiar arm as an indication of normal memory function.

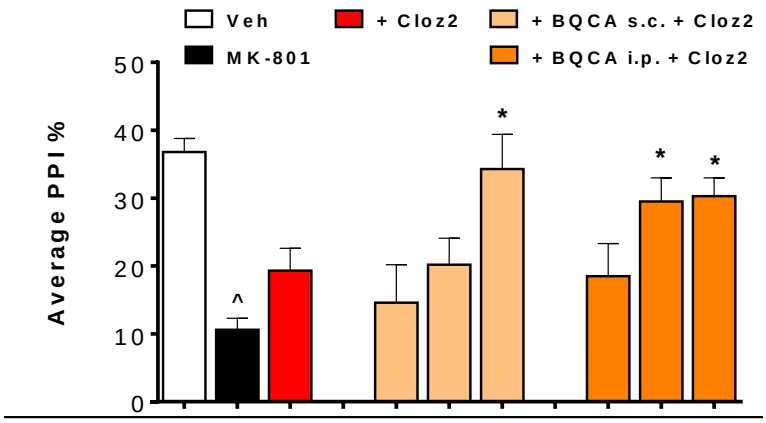


Supplemental Fig 4.

Assessment of BQCA exposure in mouse brain in the absence or presence of antipsychotics. (a) *Experiment 1*: Unbound concentrations ($C_{unbound}$ in nM) of BQCA in brains of C57Bl/6J mice after s.c. (Suspensions in 50% Pharmasolve® based vehicle) or i.p.

(Solutions in 15% DMSO based vehicle) injection. I.p. injection of BQCA resulted in a higher drug delivery to the brain than s.c. injection. (b) *Experiment 2*: $C_{unbound}$ of BQCA in $M_1^{+/+}$ and $M_1^{-/-}$ mice (C57Bl/6N background) compared to C57Bl/6J mice, suggests that BQCA brain exposure was lower in the C57Bl/6N mice than C57Bl/6J mice, and that there was no difference between the genotypes ($M_1^{+/+}$ vs. $M_1^{-/-}$). (c) *Experiment 3*: $C_{unbound}$ of BQCA in brain was assessed in C57Bl/6J mice after s.c. injection of BQCA (20 mg/kg), with or without coadministration of haloperidol (0.25 mg/kg i.p.), clozapine (2 mg/kg i.p.) or aripiprazole (top left graph). C_{total} of the antipsychotics (haloperidol, clozapine or aripiprazole) in brain was also assessed with or without coadministration of BQCA (20 mg/kg s.c.) (top right and bottom graphs). There was no enhancement of brain exposure of BQCA or antipsychotics when mice were dosed with both compounds.

$C_{unbound}$ was calculated as $C_{total} * f_u$ using published f_u values of 0.126 in brain, respectively (Gould et al., 2015). Each value represents the mean within the range of data of *n = 2-3* animals per group.



Route of administration	s.c.	s.c.	s.c.	i.p.	i.p.	i.p.
Injection dose (mg/kg)	3	10	20	0.1	1	20
nM @ 45min	22.5	30.7	36.0	na	39.1	477.4

Supplemental Fig. 5.

Reversal of disrupted PPI induced by MK-801 in C57Bl/6J mice receiving BQCA via i.p. administration using a lower dosing range, as BQCA administrated via .i.p. route (DMSO containing vehicle) allowed a higher brain exposure compared to s.c. route. There was a similar $C_{unbound}$ (in nM) of BQCA in the brain observed between BQCA administrated via i.p. and s.c. (39.1 ± 4.6 nM and 36.0 ± 4.5 nM respectively). Furthermore, a high dose of BQCA via i.p. (20 mg/kg) also produced a similar reversal effect on MK-801 treatment.

Methods and data analysis: A separated cohort of C57Bl/6J received BQCA via i.p. route, together with vehicle and MK-801 alone controls. For data analysis, this PPI data was combined with data obtained from BQCA (s.c.) + clozapine cohort (Fig. 3b). The PPI% of both vehicle groups were approximately the same: BQCA s.c. cohort = 36.7 ± 2.5% and BQCA i.p. cohort = 36.9 ± 3.2%. ANOVA test were performed on the combined PPI data. BQCA ($p < 0.001$), clozapine ($p = 0.022$) and MK-801 ($p < 0.001$) effects were found, and then followed with Tukey's post hoc comparisons, where ^ $p < 0.05$ for significant PPI disruptive effect of MK-801, and * $p < 0.05$ for difference with MK-801.