Cellular/Molecular

# Amphetamine Potentiates the Effects of β-Phenylethylamine through Activation of an Amine-Gated Chloride Channel

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**β-Phenylethylamine (βPEA) is a trace amine present in the CNS of all animals tested to date. However, its function is still not fully understood. βPEA has been suggested to function as a neurotransmitter and/or to mimic the effect of amphetamine (Amph). In support of the latter is the observation that βPEA and Amph produce similar but not identical behaviors. Here, we show that βPEA, like Amph, activates the dopamine transporter and the amine-gated chloride channel LGC-55 to generate behaviors in *Caenorhabditis elegans*. However, although Amph-induced behaviors occurred gradually during 10 min of treatment, βPEA induced maximal effects within 1 min. *In vitro* data demonstrate that βPEA activates the LGC-55 more efficiently than Amph ($K_m$ = 9 and 152 μM, respectively) and generates saturating currents that are 10 times larger than those produced by Amph. These results suggest that activation of LGC-55 mostly accounts for the behavioral effects reached after 1 min of treatment with βPEA. Importantly, our *in vitro* and *in vivo* data show that Amph increases the effects induced by βPEA on the LGC-55, indicating that Amph potentiates the effects generated by the biogenic amine βPEA. Together, our data not only identify a new target for βPEA, but also offer a novel mechanism of action of Amph. In addition, our results highlight *C. elegans* as a powerful genetic model for studying the effects of biogenic and synthetic amines both at the molecular and behavioral levels.**

*Key words:* **β-phenylethylamine; amphetamine; *Caenorhabditis elegans*; dopamine transporter; ligand-gated ion channels**

## Introduction

Phenylethylamines constitute a large class of both biogenic and synthetic compounds. Among the synthetic subgroup, amphetamine (Amph) is well known for its stimulant effects. The biogenic subgroup is comprised of well characterized neurotransmitters such as dopamine (DA), norepinephrine and serotonin, and neurotransmitters broadly named trace amines (TAs), which include β-phenylethylamine (βPEA). In the mammalian brain, βPEA is heterogeneously distributed, with the highest levels found in the nigrostriatal and mesolimbic regions (Paterson et al., 1990). These same areas are vastly innervated by dopaminergic neurons and are sites of action of Amph. βPEA is thought to enhance dopaminergic transmission, yet its specific mechanism of action remains uncertain. Changes in βPEA metabolism have been found in neurological disorders including schizophrenia and attention deficit hyperactivity disorder (ADHD), suggesting the involvement of this amine in the pathophysiology of monoaminergic systems (Boulton, 1980).

Previous studies showed that βPEA inhibits the uptake and promotes the release of the monoamines DA, norepinephrine, and, to a lesser extent, serotonin. The potency of βPEA in increasing the concentration of these neurotransmitters is comparable to that of Amph (Nakamura et al., 1998). When applied exogenously, βPEA elicits Amph-like psychostimulant responses (Bergman et al., 2001). Interestingly, the stimulant effects generated by βPEA are transient compared with those generated by Amph and, like Amph, βPEA releases DA in a manner dependent on the presence of an intact DA transporter (DAT; Sotnikova et al., 2004; Hossain et al., 2013). Subsequent experiments demonstrated that a subset of behavioral responses to βPEA were independent from DAT, suggesting that βPEA acted on other unidentified targets (Sotnikova et al., 2005). In this study, we used the model organism *Caenorhabditis elegans* to investigate the effects of βPEA and Amph in both *in vivo* and *in vitro* settings. We show that βPEA requires the amine-gated chloride channel LGC-55 to generate behaviors distinct from those induced by Amph. In fact, βPEA induced maximal behavioral effects within 1 min of treatment, whereas Amph required at least 10 min to generate the same effects. Our *in vitro* data show that βPEA actives the LGC-55 channels more efficiently than Amph ($K_m$ = 9 and 152 μM, respectively; Safratowich et al., 2013) and generates larger currents than Amph (3.7 and 0.4 μA, respectively; Safratowich et al., 2013). We suggest that these differences explain the diverse effects observed *in vivo*; that is, the faster onset of βPEA-

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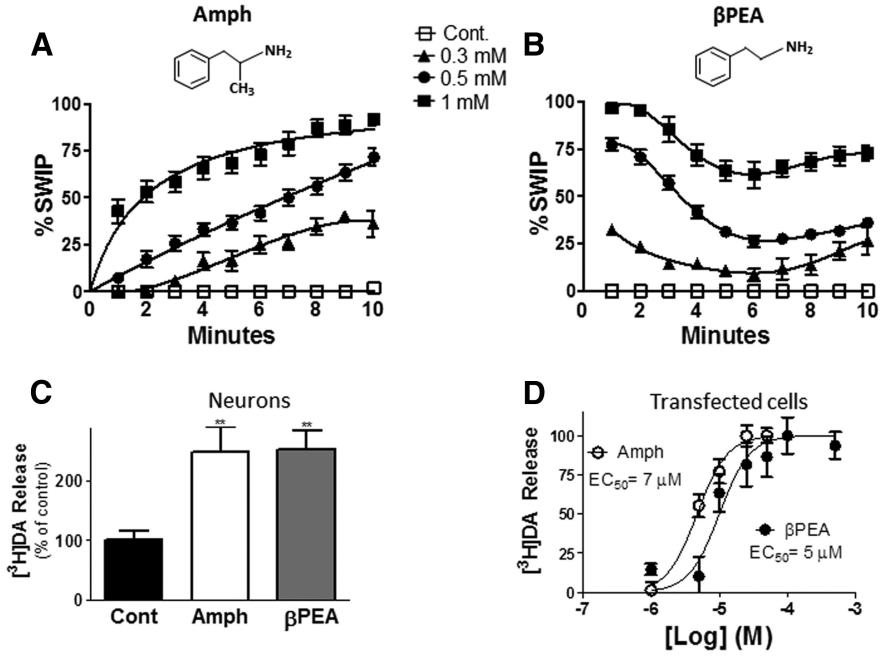


**Figure 1.** *In vivo* and *in vitro* effects of βPEA and amphetamine treatments in *C. elegans*. ***A***, In WT animals 0, 0.3, 0.5, and 1 mM Amph caused 0%, 36%, 65%, and 92% SWIP, respectively, after 10 min of treatment. These results are reproduced with permission from Safratowich et al. (2013). ***B***, Concentrations of 0, 0.3, 0.5, and 1 mM βPEA induced 0%, 32%, 77%, and 96% SWIP after 1 min; 0%, 14%, 41%, and 72% SWIP after 4 min; and 0%, 26%, 36%, and 73% after 10 min, respectively. All βPEA concentrations tested after 1 min induced SWIP levels that were statistically different with respect to SWIP in control-treated animals ($p = 0.001$, two-way ANOVA with Bonferroni's posttest). The number of animals (*n*) tested in the control (0 mM βPEA), 0.3, 0.5, and 1 mM βPEA groups were 179, 66, 285 and 92, respectively. ***C***, βPEA and Amph caused similar [$^3$H]DA release in *C. elegans* cultured neurons. Data are the average of three independent experiments. ***D***, The $EC_{50}$ for [$^3$H]DA release measured in DAT-1-transfected LLC-pk1 cells did not show statistical differences between βPEA and Amph treatments (Student's *t* test). The experiment was repeated three times and, during each experiment, concentrations were replicated in three wells.

induced behavioral effects with respect to Amph. Importantly, both our *in vitro* and *in vivo* results demonstrate that Amph potentiates the activation of the LGC-55 channels by βPEA. Therefore, our data identify a new target for βPEA and support a novel mechanism of action of Amph.

## Materials and Methods

C. elegans *strains and behavioral assays.* Nematode husbandry and swimming-induced paralysis (SWIP) assays were performed as described in Safratowich et al. (2013). Wild-type (WT; Bristol N2) and knock-out (KO) strains *dat-1(ok157)III*, *cat-2(e1112)II*, *dop-1(vs100)X*, *dop-2(vs105)V*, *dop-3(ok295)X*, *dop-4(tm1392)X*, *ser-2(pk1357)X*, *ser-3(ok1995)I*, *ser-4(ok512)III*, *tyra-3(ok325)X*, *lgc-53(n4330)X*, and *lgc-55(n4311)V* were obtained from the *C. elegans* Genetics Center at the University of Minnesota (Minneapolis). Rescue animals lgc-55(tm2913); lin-15(n765ts; zfEx42 [pglr-1::LGC-55] were kindly donated by Dr. Mark Alkema (University of Massachusetts–Worcester). At least 60 animals were tested in each group in at least five independent trials. The exact number of animals used per group is shown in the figure legends. Behavioral data were analyzed statistically using one-way ANOVA with Bonferroni's multiple-comparison test unless otherwise indicated.

*Oocyte expression and electrophysiology.* Complementary RNAs (cRNA) synthesis, oocyte injection, and TEVC experiments were performed as described in Safratowich et al. (2013). Figure 1*A* is used with permission from Safratowich et al. (2013) and is provided here as visual for a direct comparison with βPEA data.

[$^3$*H*]*DA release assays in* C. elegans *primary cultures and transfected cells.* We prepared *C. elegans* primary cultures as described in Carvelli et al. (2004). Two-day-old embryonic cells ($10^6$ cells/well) were preloaded with 5 nM [$^3$H]DA for 30 min at room temperature. Cells were washed five times and then 100 μM βPEA or Amph was applied for 1 min. Samples were collected and counted for radioactivity. $EC_{50}$ values were calculated in LLCpk1 cells transfected with 0.5 μg of *C. elegans* DAT (DAT-1) cDNA and maintained in EMEM with 5% FBS, 100 U/ml penicillin, and 100 mg/ml streptomycin. Cells were preincubated with 20 nM [$^3$H]DA and treated with 0.001–0.5 mM βPEA or Amph. Nisoxetine (100 μM) was used to calculate specific release because it was shown previously to inhibit [$^3$H]DA uptake ($K_i = 3$ nM) in DAT-1-transfected cells (Jayanthi et al., 1998).

## Results

### βPEA- and amphetamine-induced behaviors in *C. elegans*

We showed previously that increased extracellular DA levels generated a potent inhibitory effect on the ability of *C. elegans* to swim. We named this behavior SWIP. Genetic ablation (McDonald et al., 2007) or pharmacological blockage of DAT-1 (Carvelli et al., 2008) was sufficient to cause SWIP. Not surprisingly Amph, which is a DAT substrate and a DA releaser, also induced SWIP (Carvelli et al., 2010; Safratowich et al., 2013). In fact, animals treated with 0.3–1 mM Amph exhibited SWIP within 10 min (Fig. 1*A*, used with permission from Safratowich et al., 2013). Here, we found that animals challenged with 0.3–1 mM βPEA exhibited SWIP in a dose-dependent manner (Fig. 1*B*). However, whereas Amph maximal effect occurred after 10 min, βPEA caused maximal SWIP within a few seconds. Moreover, the maximal SWIP levels reached with Amph lasted until Amph was washed out, whereas the maximal SWIP levels generated by βPEA decreased over time despite the sustained presence of the drug. This time-dependent decrease of βPEA-induced SWIP was inversely proportional to the concentration of βPEA used. For example, when treated with 0.5 mM βPEA, 71 ± 2% of animals recovered from SWIP after 6 min (Fig. 1*B*, ●), whereas with 1 mM βPEA, only 36 ± 4% animals recovered from SWIP (Fig. 1*B*, ■), suggesting that the decrease of SWIP is specifically linked to βPEA treatment. Together, these results demonstrate that the kinetics for βPEA-induced SWIP are distinct from those of Amph.

We have also shown previously that Amph-induced SWIP is caused in part by an increase of extracellular DA released through DAT-1 (Carvelli et al., 2010). To determine whether the difference in the extent of SWIP between βPEA and Amph at 1 min was caused by elevated DA release, we compared the ability of βPEA and Amph to increase the extracellular levels of DA. Cultured *C. elegans* DA neurons were preloaded with [$^3$H]DA and then treated with βPEA or Amph for 1 min. Both drugs induced significant increases of extracellular [$^3$H]DA with respect to controls (253 ± 31% and 248 ± 41%, respectively; $^{**}p = 0.003$, one-way ANOVA with Bonferroni's posttest), but no difference was observed between βPEA and Amph treatments (Fig. 1*C*). Moreover, the $EC_{50}$ calculated for DA release induced by βPEA (5 ± 0.05 μM) and Amph (7 ± 0.08 μM) in DAT-1-transfected cells revealed no significant difference (Student's *t* test; Fig. 1*D*). These results suggested that the higher SWIP rates measured with

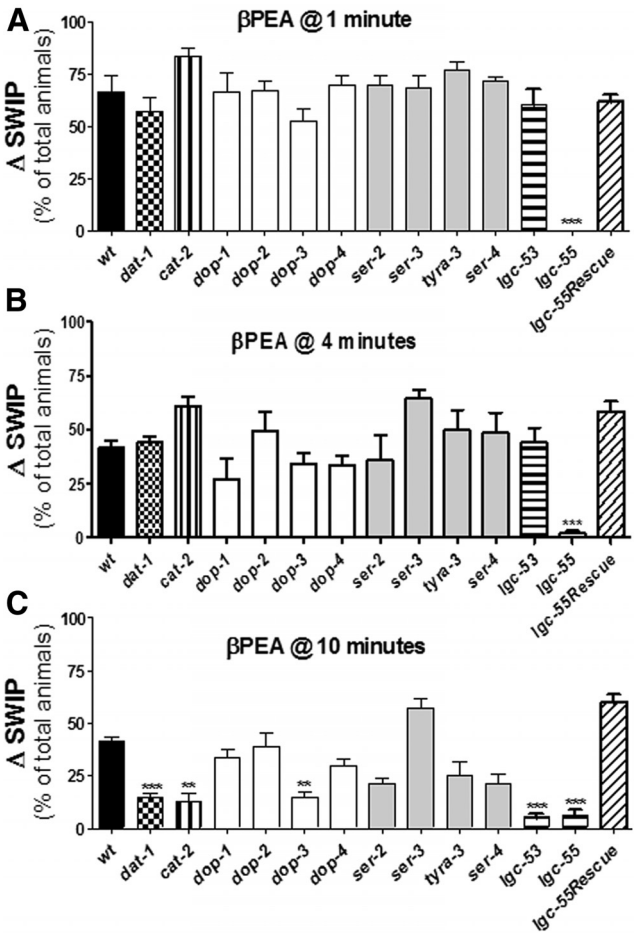


**Figure 2.** Proteins required for βPEA-induced SWIP at different time points. DAT-1 (*dat-1*), DA (*cat-2*), DA receptors (*dop-1*, *dop-2*, *dop-3*, *dop-4*), trace amine receptors (*ser-2*, *ser-3*, *tyra-3*, *ser-4*), and the amine-gated channel *lgc-53* KOs did not exhibit statistically differences in SWIP with respect to WT after 1 (***A***) and 4 (***B***) minutes of 0.5 mM βPEA treatment (one-way ANOVA with Bonferroni's posttest). The LGC-55 KO (*lgc-55*) showed 100% and 95% reduction in SWIP after 1 (***A***) and 4 min (***B***) of treatment, respectively. ***C***, After 10 min, *dat-1*, *cat-2*, *dop-3*, *lgc-53*, and *lgc-55* KOs exhibited significant reductions in SWIP with respect to WT animals (***$p = 0.0001$; **$p = 0.001$, one-way ANOVA with Bonferroni's posttest). *lgc-55* expression driven by the *glr-1* promoter in *lgc-55* KO animals rescued the βPEA-induced SWIP phenotype after 1, 4, and 10 min. As the *dat-1* KOs showed basal SWIP, we reported the ΔSWIP as the number of paralyzed animals upon βPEA treatment minus the number of paralyzed animals upon vehicle treatment. In ***A–C***, mutants were compared with WT animals using one-way ANOVA with Bonferroni's posttest. The numbers of animals per each group were as follows: WT = 98, *dat-1* = 67, *cat-2* = 69, *dop-1* = 66, *dop-2* = 60, *dop-3* = 69, *dop4* = 60, *ser-2* = 69, *ser-3* = 63, *tyra-3* = 65, *ser-4* = 62, *lgc-53* = 68, *lgc-55* = 67, and *lgc-55Rescue* = 127.

βPEA are not caused by the ability of βPEA to release larger amounts of DA with respect to Amph.

### βPEA induces behaviors independently from DAT, DA, and TA receptors

We showed previously that Amph-induced SWIP requires a functional DAT-1 (Carvelli et al., 2010; Safratowich et al., 2013). To investigate whether βPEA-induced SWIP was dependent on DAT-1, we measured βPEA-induced SWIP in DAT-1 KO animals (*dat-1*) after 1 min of treatment. We found that βPEA caused similar levels of SWIP in *dat-1* compared with WT animals (Fig. 2*A*). Next, we tested whether DA itself was involved in generating the high SWIP levels induced by βPEA after 1 min. We measured βPEA-induced SWIP in *cat-2* KO animals that lack tyrosine hydroxylase, a key enzyme for DA synthesis (Sanyal et al., 2004) and no difference was found with respect to WT (Fig. 2*A*). We then investigated the possibility that βPEA itself binds directly to DA receptors and induces fast SWIP. However, in animals with the D1-like (*dop-1*, *dop-4*) or D2-like (*dop-2*, *dop-3*) DA receptors knocked out (Suo et al., 2002; Chase and Koelle, 2007; Sugiura et al., 2005), we found no difference in βPEA-induced SWIP with respect to WT animals (Fig. 2*A*). Together, these data demonstrate that the DAT-1, DA, and DA receptors are not required to generate βPEA-induced SWIP after 1 min of treatment.

Next, we investigated whether βPEA activated the TA receptors to generate high SWIP rates. We measured βPEA-induced SWIP in animals lacking the tyramine SER-2 (Rex and Komuniecki, 2002), the octopamine SER-3 (Suo et al., 2006), the tyramine/octopamine TYRA-3 (Wragg et al., 2007), and the serotonin receptor SER-4 (Hamdan et al., 1999) and the knock-out of these TA receptors did not affect βPEA-induced SWIP after 1 min of treatment (Fig. 2*A*). To conclude, these data demonstrate that the high SWIP levels measured after 1 min of βPEA treatment do not require DA or DAT-1, suggesting that βPEA itself may be a neurotransmitter acting on targets other than DA and TA receptors.

### βPEA-induced behaviors require LGC-55 channels

Two amine-gated chloride channels, LGC-53 and LGC-55, have been identified in *C. elegans* by Ringstad et al. (2009) and Pirri et al. (2009). Both groups showed strong expression of LGC-55 in several head neurons and in neck muscle cells. We demonstrated recently that Amph-induced SWIP depends on both DAT-1 and the LGC-55 (Safratowich et al., 2013). Given the chemical structure similarities between Amph and βPEA (Fig. 1*A*,*B*), we reasoned that the LGC-55 and/or LGC-53 receptors might be activated by βPEA to generate high rates of SWIP. In the LGC-53 KO animals (*lgc-53*), βPEA-induced SWIP levels were equivalent to those observed in WT (Fig. 2*A*), suggesting that these channels are not required to generate SWIP after 1 min of βPEA treatment. In contrast, when LGC-55 KO animals (*lgc-55*) were challenged with βPEA, we did not observe SWIP after 1 min of treatment (***$p = 0.0001$, one-way ANOVA with Bonferroni's posttest; Fig. 2*A*). These results support that LGC-55 channels are required to generate βPEA-induced SWIP. We also investigated the effect of βPEA at later time points in WT, *lgc-53*, and *lgc-55* (Fig. 2*B*) and found that, after 4 min, only the *lgc-55* animals showed significantly reduced SWIP with respect to WT (***$p = 0.0001$, one-way ANOVA with Bonferroni's posttest). However, after 10 min of treatment (Fig. 2*C*), a strong reduction in SWIP was measured in both *lgc-55* and *lgc-53* (88 ± 2% and 85 ± 3%, respectively; ***$p = 0.0001$, one-way ANOVA with Bonferroni's posttest). Interestingly, SWIP could be fully rescued when *lgc-55* cDNA fused to *glr-1* promoter was introduced into the *lgc-55* KO animals (Pirri et al., 2009; Fig. 2*A–C*). This demonstrates that the expression of LGC-55 and its functional complementation in neurons, but not in muscle cells, is required to generate SWIP. In fact, the *glr-1* promoter, which encodes an AMPA-like ionotropic glutamate receptor, drives *lgc-55* expression only in 17 *C. elegans* neurons, including motoneurons (Hart et al., 1995; Maricq et al., 1995). The *lgc-55*-rescued animals, like WT animals (Fig. 1*A*,*B*), did not show SWIP when tested in control solution. In fact, after 1, 4, and 10 min of exposure to control solution, only 0%, 0%, and 1.6 ± 1% animals exhibited SWIP, respectively. Together, these data demonstrate that neuronally expressed LGC-55 receptors are needed to generate βPEA-induced SWIP, whereas the LGC-53 receptors are recruited only at later time points.

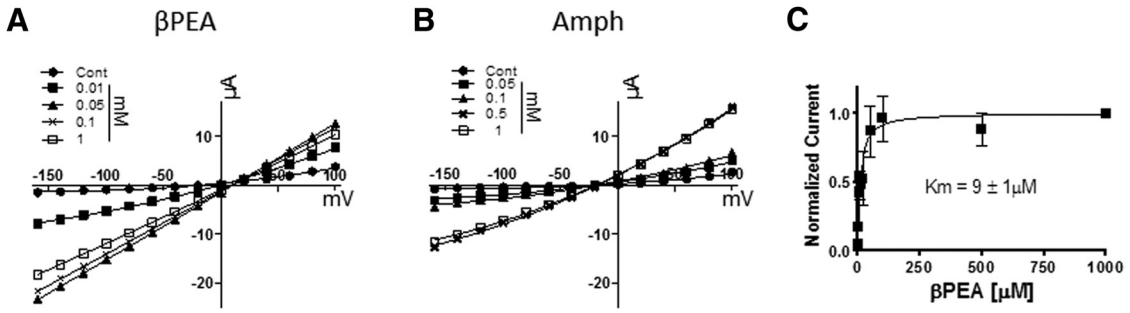


**Figure 3.** βPEA activates the LGC-55 channels more efficiently than Amph. ***A***, ***B***, Representative current–voltage relationships generated in lgc-55-injected oocytes perfused with βPEA ($n = 12$) or Amph ($n = 16$), respectively. ***C***, Dose–response curve of βPEA-induced currents generated when the oocyte membrane potential was held at −60 mV ($n = 12$). Currents were normalized to the maximal current value and fitted to a Michaelis–Menten equation.

### After prolonged treatments, βPEA-induced SWIP involves proteins other than LGC-55

Our results (Fig. 1*B*) indicate that 0.3–1 mM βPEA induces high levels of SWIP within the first minute of treatment, with reduction of SWIP after 3–6 min, followed by a slight increase of SWIP at minutes 8–10. To investigate the basis for these kinetics, we performed SWIP assays after 4 and 10 min in mutant animals. We found that, after 4 min (Fig. 2*B*), the DA and TA receptor CAT-2 and DAT-1 KOs exhibited no significant differences in SWIP with respect to WT animals (one-way ANOVA with Bonferroni's posttest). Interestingly, though, after 10 min (Fig. 2*C*), only animals lacking the DAT-1, DA (*cat-2*) and the DA receptor DOP-3 exhibited significant SWIP reduction with respect to WT (66 ± 2%, 68 ± 2%, and 66 ± 3%, respectively; $^{**}p = 0.001$ and $^{***}p = 0.0001$, one-way ANOVA with Bonferroni's posttest). These results demonstrate that, after 10 min, βPEA likely recruits the same key players as Amph to induce SWIP (Carvelli et al., 2010; Safratowich et al., 2013).

Collectively, the experiments shown in Figure 2 demonstrate that only the LGC-55 receptors, which are expressed in GLR-1-expressing neurons, are required to generate βPEA-induced SWIP within 1 min.

### βPEA activates the LGC-55 channels directly

Our data demonstrate that LGC-55 is needed by βPEA to generate SWIP within a few minutes of treatment (Fig. 2*A*,*B*). Therefore, we investigated whether βPEA, like Amph (Safratowich et al., 2013), activates the LGC-55 directly. We performed two electrode voltage-clamp experiments in *Xenopus* oocytes injected with lgc-55 cRNA and found that increasing βPEA concentrations evoked currents in a dose–response manner (Fig. 3*A*). Interestingly, 0.01–1 mM βPEA generated larger currents than equivalent concentrations of Amph (cf. Fig. 3*A*,*B*). Indeed, the $K_m$ calculated for βPEA-induced currents (Fig. 3*C*) was 17 times lower than the $K_m$ previously calculated for Amph-induced currents (152 ± 29 μM; Safratowich et al., 2013). Moreover, a saturating concentration of βPEA gave a current (3.7 ± 0.6 μA) larger than that seen previously with a saturating concentration of Amph (0.4 ± 0.09 μA; Safratowich et al., 2013). Therefore, the comparison between βPEA and Amph efficacy reveals that βPEA is more potent at LGC-55 because it activates the receptor at lower concentrations and produces larger currents than Amph.

### Amph potentiates the effects of βPEA on the LGC-55 channels

Given the different efficiencies of Amph and βPEA to activate LGC-55, we investigated whether Amph interfered with βPEA in activating the LGC-55. We perfused Amph alone (Fig. 4*A*,*B*, I-II) and together with βPEA (Fig. 4*A*,*B*, II-III) onto oocytes expressing LGC-55. Amph perfusion was discontinued to measure βPEA-induced currents (Fig. 4*A*,*B*, III-IV). Interestingly, we found that βPEA-induced currents were potentiated in presence of Amph. Indeed, 1 μM βPEA generated currents of 15 ± 2 μA, whereas 1, 5, and 10 μM Amph generated currents of 1.3, 2, and 4 μA, respectively (Fig. 4*C*). However, when 1 μM βPEA was perfused together with 1, 5, or 10 μM Amph, we measured currents of 42, 30, and 26 μA, respectively (Fig. 4*C*). Interestingly, the potentiation effect of 1 μM Amph was significantly higher than that measured with 10 μM Amph ($^{*}p = 0.01$, one-way ANOVA with Bonferroni's posttest). Finally, we investigated whether Amph could modulate the *in vivo* activity of the LGC-55 during βPEA stimulation. We cotreated animals with Amph/βPEA and found that, after 1 min, they exhibited 66 ± 5% SWIP, whereas when applied separately, the two drugs generated 3 ± 2% and 32 ± 5% SWIP, respectively (Fig. 4*D*). These results demonstrate that Amph potentiates βPEA-induced effects *in vivo*. In fact, Amph/βPEA-

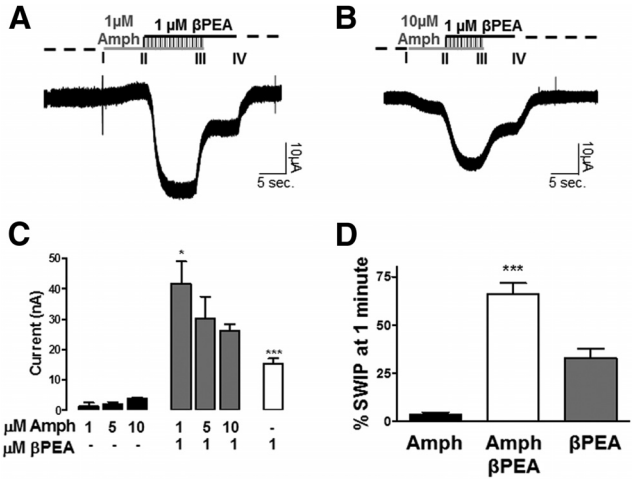


**Figure 4.** Amph potentiates the LGC-55 activation by βPEA. Representative recordings of 10 LGC-55-expressing oocytes perfused with 1 μM (***A***) or 10 μM (***B***) Amph alone (I-II). Subsequently, 1 μM βPEA was added together with 1 or 10 μM Amph (II-III). Amph was removed and βPEA was perfused alone (III-IV). ***C***, Average of currents as measured in ***A*** and ***B***. Currents measured during Amph/βPEA coperfusion were statistically higher than those obtained with βPEA alone ($^{***}p \leq 0.0001$, one-way ANOVA with Bonferroni's posttest). Coperfusion of 1 μM Amph generated a higher potentiation of βPEA-induced currents with respect to 10 μM Amph ($^{*}p = 0.03$, one-way ANOVA with Bonferroni's posttest). ***D***, After 1 min, animals cotreated with 500 μM Amph and 300 μM βPEA ($n = 92$) exhibited higher SWIP with respect to animals treated with Amph alone ($n = 65$) or βPEA alone ($n = 61$) ($^{***}p = 0.0001$, one-way ANOVA with Bonferroni's posttest).

induced SWIP was approximately twice as high as the values we expected if the effects of the two drugs were purely additive.

To conclude, these data demonstrate that both *in vitro* and *in vivo* Amph potentiates the effects induced by βPEA. Specifically, 1 μM Amph/βPEA generated currents that were 30 or 3 times larger than those generated by 1 μM Amph or βPEA alone, respectively (Fig. 4*C*). Similarly, Amph/βPEA cotreatment generated SWIP values that were 20 or 2 times higher than those generated by each individual drug, respectively (Fig. 4*D*).

## Discussion

*In vitro* and *in vivo* studies have suggested that βPEA is an endogenous psychostimulant that shares similar mechanisms of action with Amph (Gilbert and Cooper, 1983; Janssen et al., 1999). Like Amph, βPEA induces DA efflux through DAT, but generates only transient Amph-like behaviors. One explanation that has been brought forward for this difference is that βPEA is degraded more readily than Amph by the monoamine oxidase type B (MAO-B; Bergman et al., 2001). Interestingly, in DAT KO mice, which are hyperactive (Giros et al., 1996), βPEA and Amph still increased extracellular DA and produced certain stereotypes, indicating that targets other than DAT and DA are responsible for some of the behaviors generated by phenylethylaminic compounds (Carboni et al., 2001; Sotnikova et al., 2004; Sotnikova et al., 2005; Safratowich et al., 2013). Here, we have investigated the effects of βPEA and Amph both at the molecular and behavioral levels and found similarities and differences, as well as functional interactions in the mechanism of action of these two compounds.

Our previous data showed that in *C. elegans* Amph requires both key components of the dopaminergic system (DA, DAT-1, receptors) and the LGC-55 channels to generate gradual paralysis within 10 min (Carvelli et al., 2010; Safratowich et al., 2013). The present study demonstrates that βPEA acts on the same targets to affect behaviors in *C. elegans*. However, these studies uncovered distinct kinetics differences in the action of the two compounds. βPEA recruited LGC-55 within a few seconds of its application to generate maximal SWIP. The involvement of DAT-1, DA, and DOP-3 in βPEA-induced SWIP was only observed after prolonged treatments, which coincides with the temporal action of Amph (Carvelli et al., 2010). It is unlikely that these time-dependent outcomes are due to permeability differences between the two compounds, because Amph and βPEA have comparable lipophilic values (LogP = 1.8 and 1.4, respectively). Interestingly, our *in vitro* data demonstrate that βPEA activated the LGC-55 more efficiently and generated larger currents than Amph (Fig. 3), suggesting that the larger currents generated by βPEA underlie the robust behaviors generated by this compound. Together, our data support the hypothesis that βPEA acts via two different mechanisms: (1) the robust activation of the LGC-55 channels (Fig. 3), which generates high SWIP levels within a few seconds (Fig. 1*B*), followed by (2) DA efflux through DAT-1 (Fig. 1*C*,*D*), which activates the DOP-3 receptors to generate SWIP (Fig. 2*C*). These conclusions are also supported by the observation that *lgc-55* KOs did not exhibit SWIP, whereas cell-specific rescue experiments showed that reexpression of *lgc-55* cDNA in 17 classes of neurons fully restored SWIP in the *lgc-55* KO animals (Fig. 2). These results provide direct *in vivo* evidence that βPEA-activated LGC-55 receptors mediate neuronal function in *C. elegans*. Finally, we exclude the possibility that the higher SWIP levels measured with βPEA after 1 min are caused by high levels of extracellular DA, because βPEA and Amph induced similar levels of DA release in neuronal culture (Fig. 1*C*).

Similarly to mammalian systems, our data show that behaviors induced by βPEA in *C. elegans* had a short-lasting effect compared with Amph (Fig. 1*A*,*B*). The mechanism underlying these results remains unclear, although faster degradation of βPEA could be possible because MAO homologs have been identified in *C. elegans* (Weyler, 1992). However, we speculate that SWIP recovery cannot be explained by faster βPEA degradation as animals are continually immersed in a solution containing βPEA (Fig. 1*B*).

The LGC-55 channels are members of the cys-loop ligand-gated ion channel (LGIC) receptors superfamily (Pirri et al., 2009; Ringstad et al., 2009), which includes the well studied mammalian nicotinic acetylcholine, $5HT_3$, glycine, and GABA (type A and C) receptors (Sine and Engel, 2006). Previous data have shown that the activation of the LGIC is not simply a direct consequence of substrate binding, but rather is a multistep process in which binding of the ligand induces conformation changes into the so-called "flip state" from which the channel shifts into its open configuration (Farrant and Kaila, 2007; Lape et al., 2008). Full and partial agonists enable receptors to transition from the inactivated to the activated state with different efficiencies, with full agonists exhibiting a more efficient transition into the flip state. Our data show that βPEA activates the LGC-55 channels more efficiently than Amph and generates larger currents than those generated by Amph (Fig. 3), suggesting that βPEA and Amph act as full and partial agonists for LGC-55, respectively. This phenomenon, which was reported for $GABA_A$ and glycine receptors, can be potentiated by neurosteroids, benzodiazepines, anesthetics, and ethanol (Mihic et al., 1997). Similarly, our data suggest that Amph amplifies the transient behavioral effects induced by βPEA by potentiating the activity of the LGC-55 (Fig. 4). Considering that low Amph concentrations are used to treat ADHD, our observation that 1 μM Amph potentiates the effect of βPEA on LGC-55 channels might have important physiologic implications if LGC-55 homologs are present in humans. The existence of amine-gated channels in mammals has long been suggested (Yang and Hatton, 1994). More recently, Hatton and Yang (2001) demonstrated that, in the brain, histamine generates fast IPSPs through the activation of as-yet-unidentified chloride channels. In fact, these receptors are distinct from the well known ionotropic GABA and glycine receptors because they are insensitive to bicuculline or strychnine. Interestingly, we have screened the human protein database and found four orphan proteins sharing 30–45% identity with LGC-55 at the amino acid level, providing evidence that LGC-55 homologs might indeed exist in humans.

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