

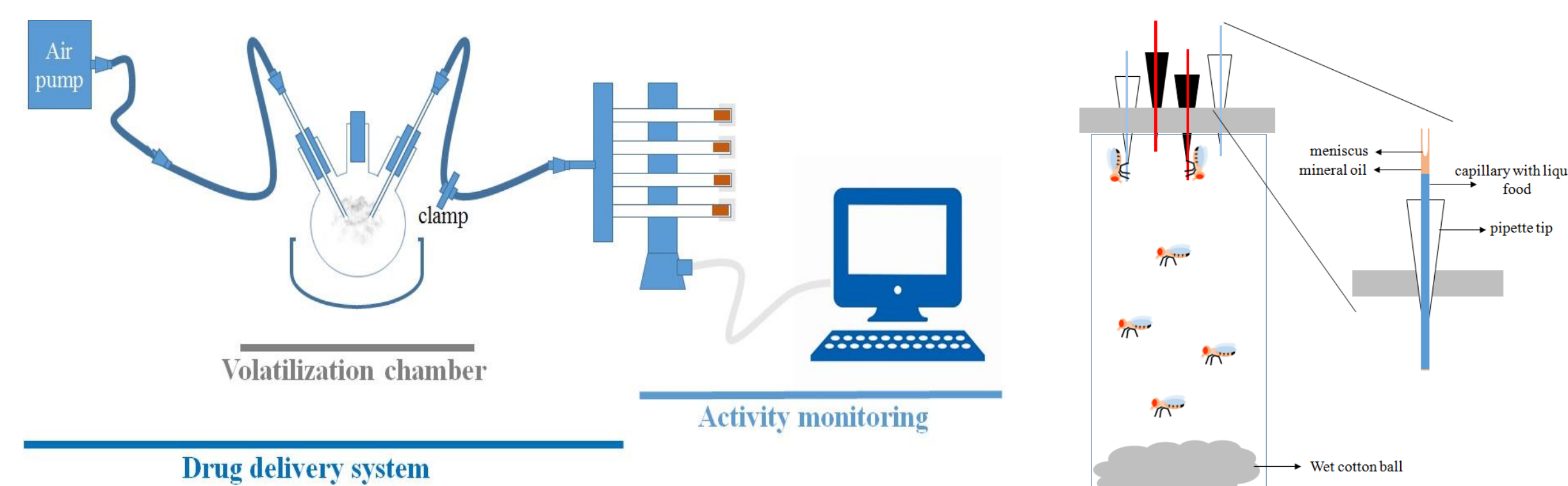
Circadian genes have phenotype-specific roles in psychostimulant-induced neuronal plasticity in *Drosophila*

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OUR INTEREST

- The best approach for identification of functional genes involved in addiction is a genetic screen for addiction-related endophenotypes in genetically tractable model organism such as *Drosophila*.
- There is a lack of highthroughput and objective tests that measure drug induced phenotypes in *Drosophila*.
- Our specific interest is in studying effects that psychostimulants cocaine (COC) and methamphetamine (METH) have on fly behavior.



APPROACH

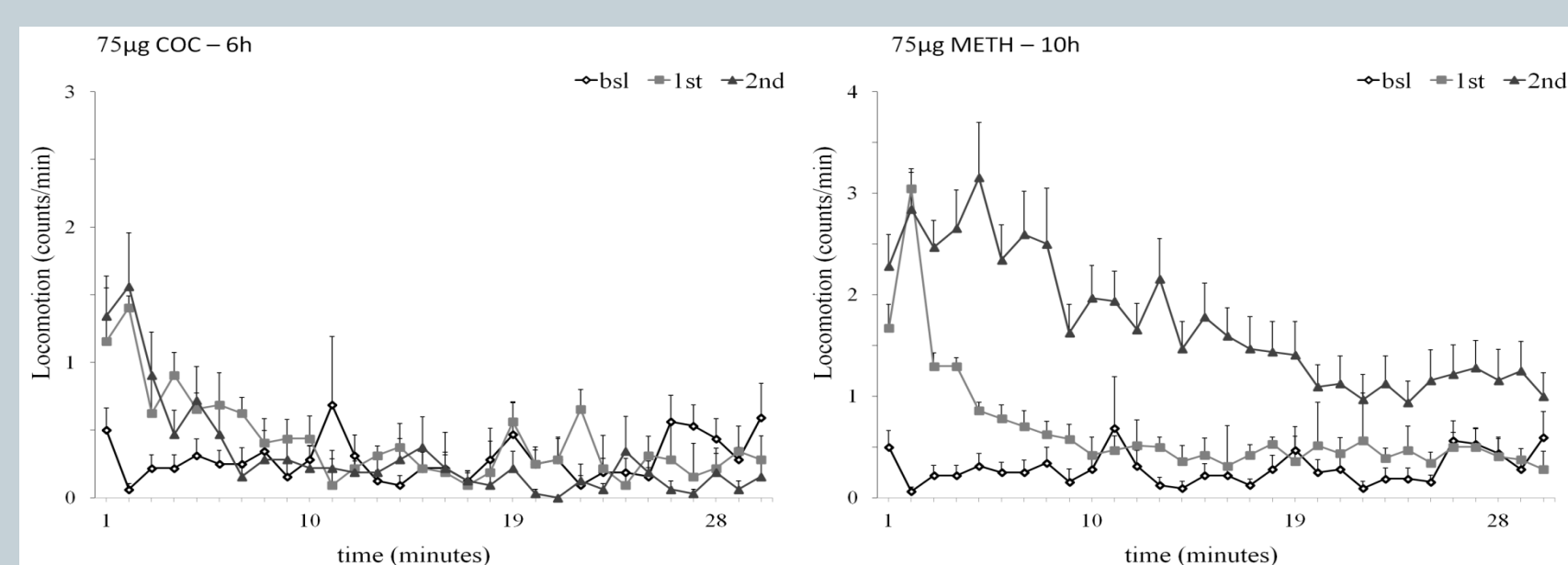
- To measure motor-activating effects of COC and METH we developed “FlyBong” for quantifying locomotor activity in response to acute and repeated administrations.
- To measure rewarding effects of COC and METH we adapted CAFÉ assay to measure preferential consumption of food with drugs over standard sugar food.
- We compare behavioral phenotypes between *wt* and circadian mutants: *Clk^{Jrk}*, *cyc⁰¹*, *per⁰¹* and *tim⁰¹*.



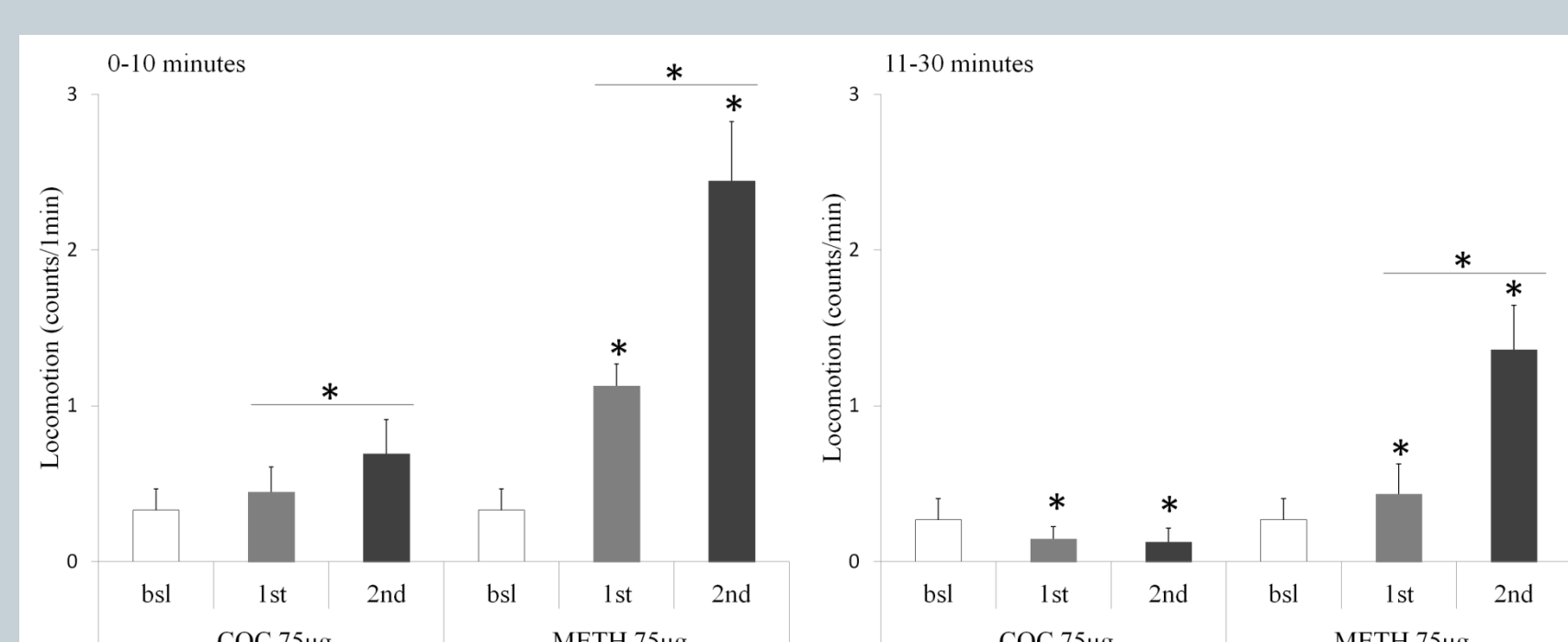
I MOTOR-ACTIVATING EFFECT OF VOLATILIZED COC AND METH

Flies respond to volatilized COC or METH with robust, but transient increase in locomotor activity – sensitivity (SENSIT).

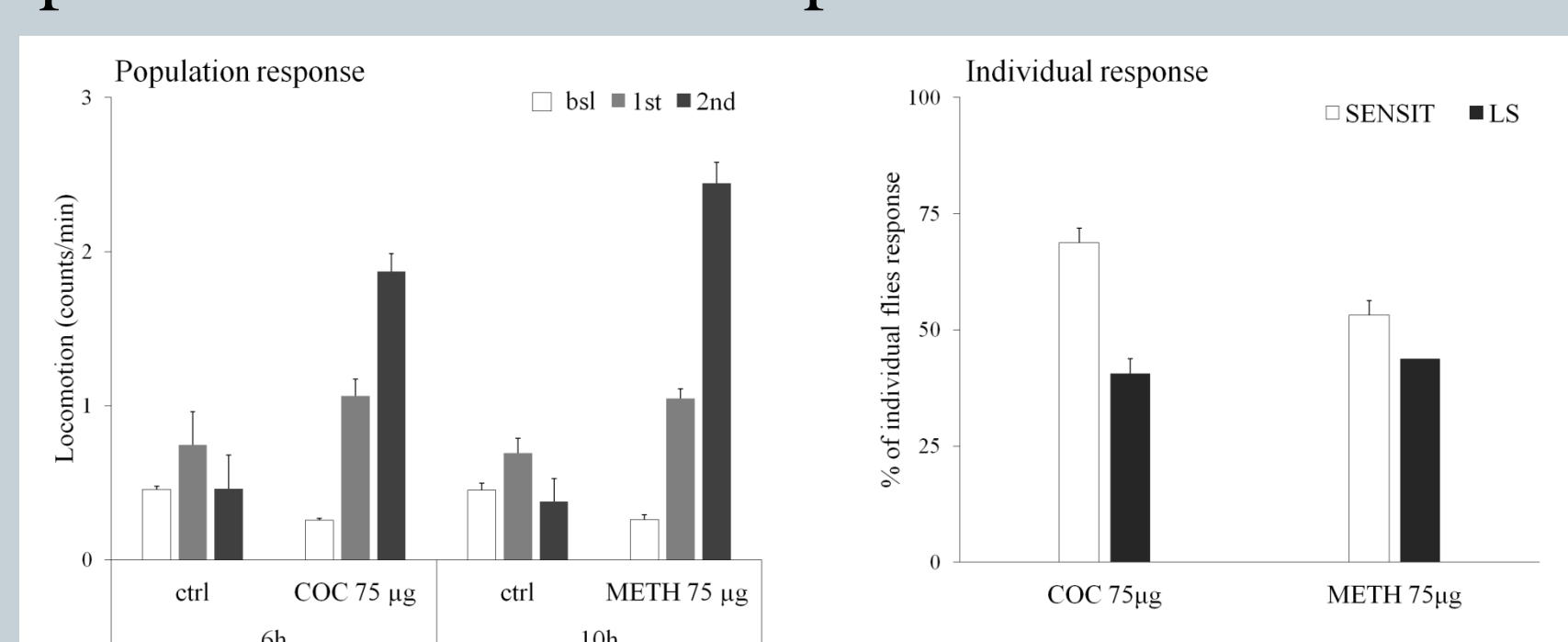
Repeated administration of the same concentration leads to further increase in locomotor activity and represents locomotor sensitization (LS).



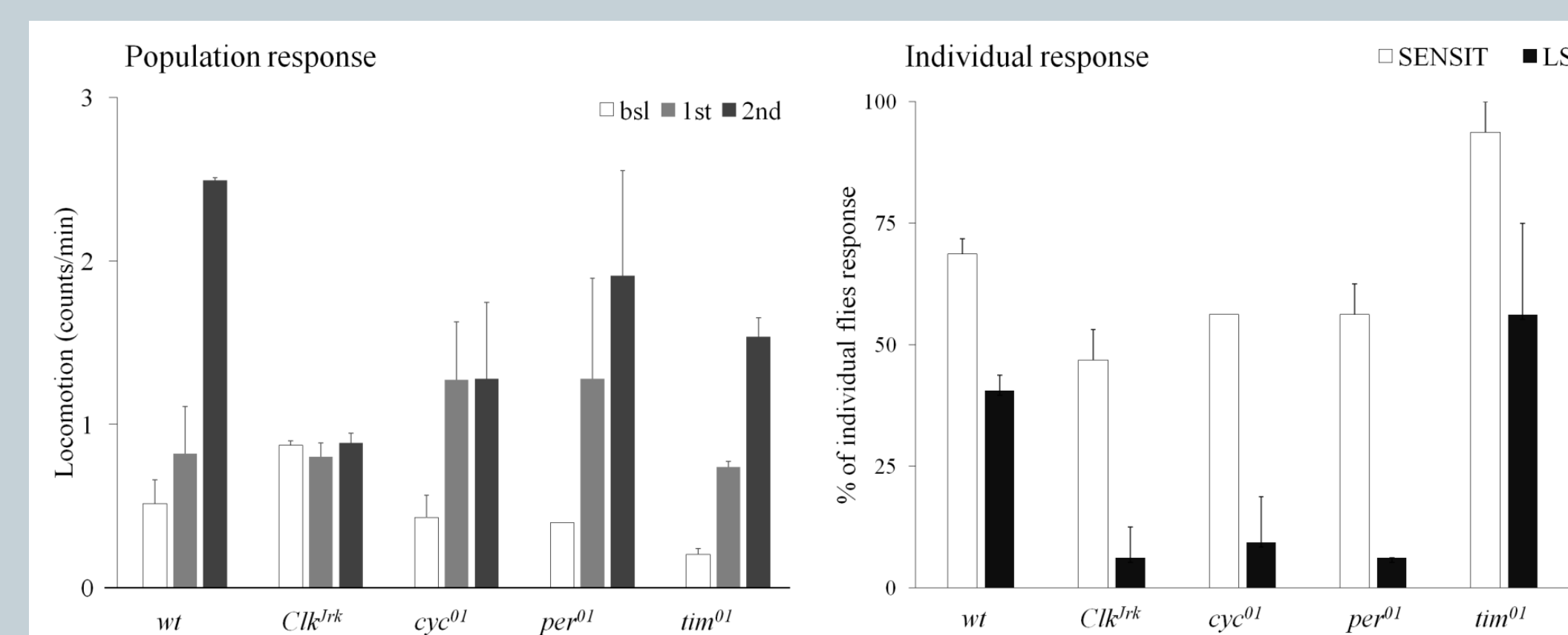
We show that flies develop LS to METH, with distinct temporal profile from COC.



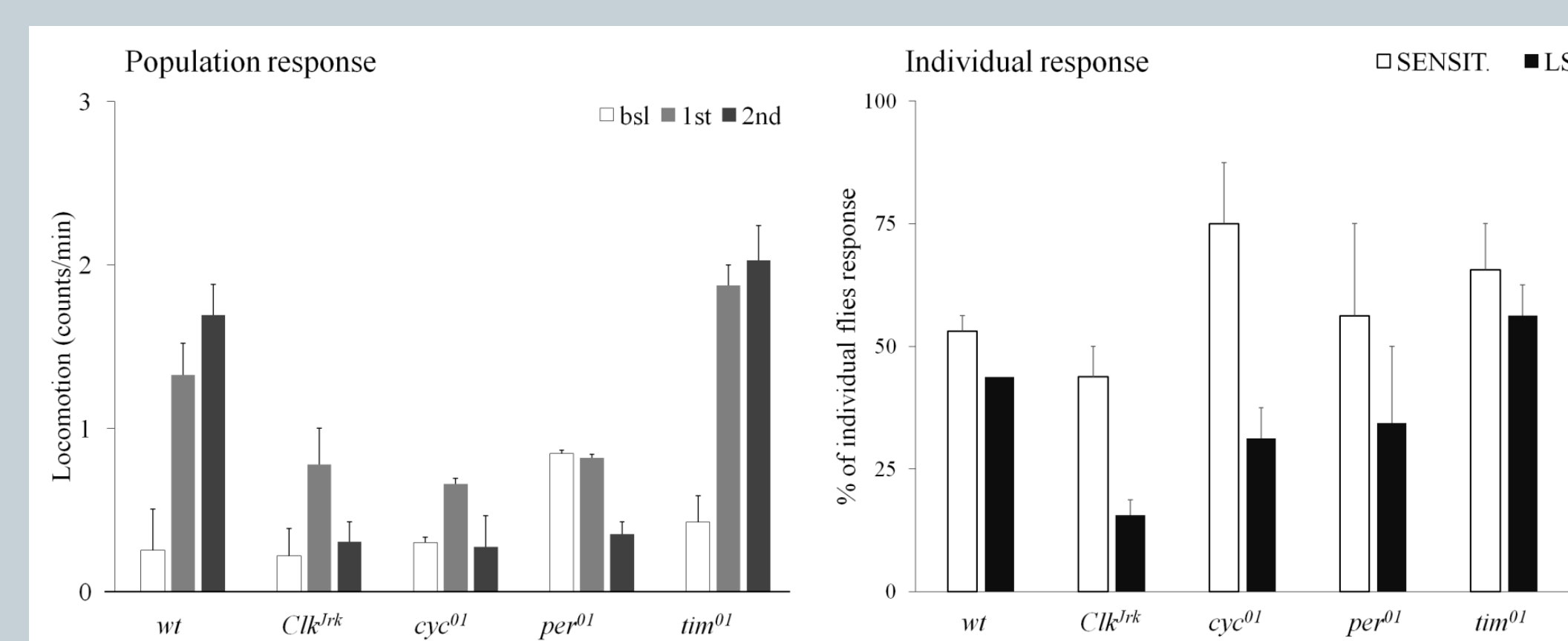
We can distinguish between average population response and individual response.



We validated FlyBong by showing that *Clk^{Jrk}*, *cyc⁰¹* and *per⁰¹* flies do not develop locomotor sensitization to volatilized COC at population or individual level.

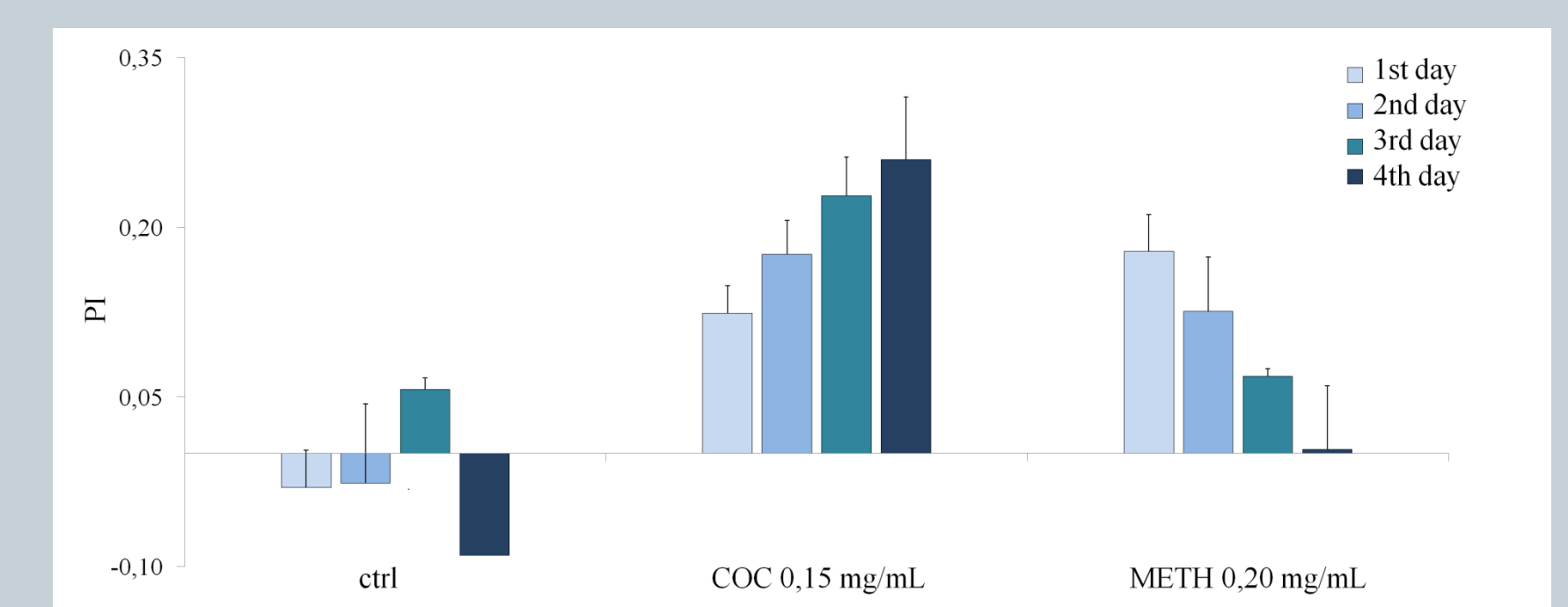


Locomotor sensitization to METH depends on *Clk^{Jrk}* gene.

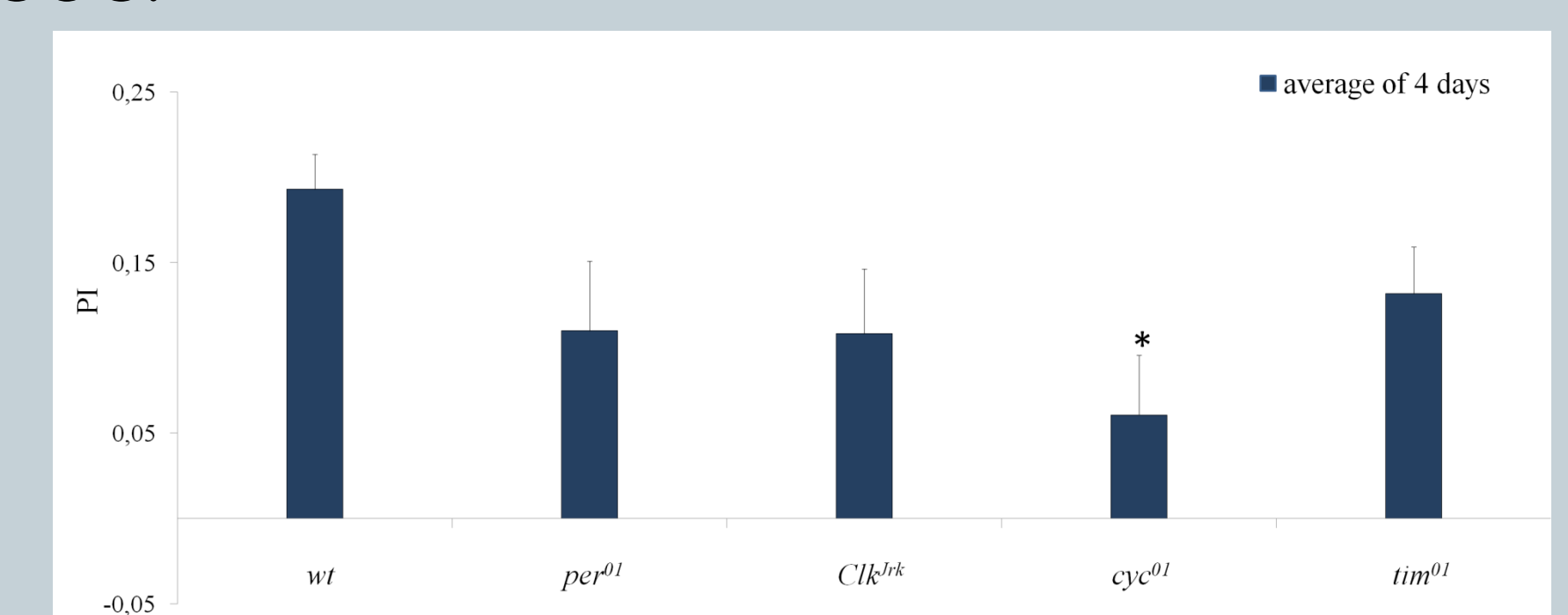


II PREFERENTIAL CONSUMPTION

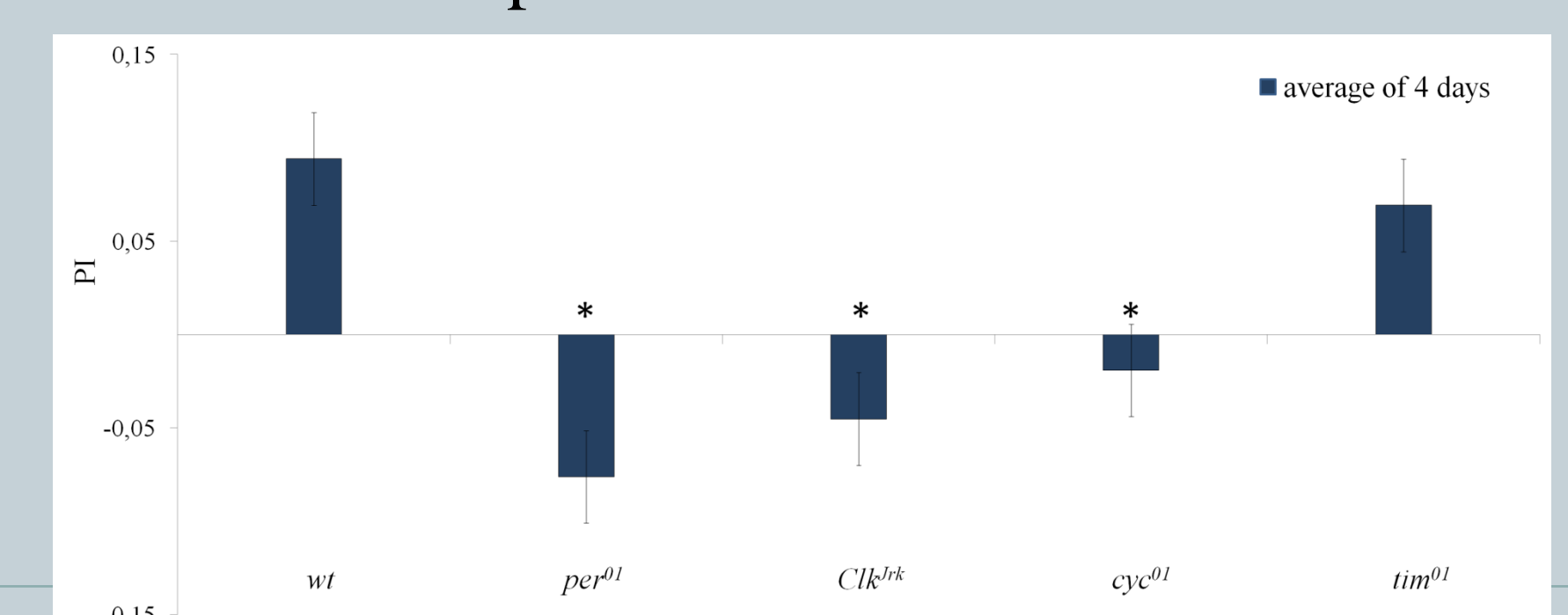
Flies preferentially consume food with COC or METH over regular sugar food, in spite of inherently bitter taste of these drugs. Flies show higher preference for consumption of COC.



Circadian gene *cyc⁰¹* modulates preferential consumption of COC.



Circadian genes *Clk^{Jrk}*, *per⁰¹* and *cyc⁰¹* modulate preferential consumption of METH.



Statistical significance $p < 0.05$

INTERPRETATION

- Locomotor response to COC and METH has several distinctive features: acute and chronic response is more transient after COC than METH administration.
- Time interval between two doses for induction of LS is different for COC and METH.
- FlyBong was validated by showing that *Clk^{Jrk}*, *cyc⁰¹* and *per⁰¹* regulate LS to COC.
- Only *Clk^{Jrk}* regulates LS to METH.
- Flies differ in preferential consumption of COC and METH.
- *cyc⁰¹* regulates preferential consumption of COC, while *Clk^{Jrk}*, *per⁰¹* and *cyc⁰¹* regulate preferential consumption of METH.

CONCLUSION

- We developed a new high throughput and objective method for measuring locomotor activity induced by exposure to volatilized substances that can be used for a genetic screen.
- We adapted CAFÉ assay for measuring rewarding effects of psychostimulants represented as preferential consumption.
- COC and METH have distinct phenotypes in both tests, which can be used for dissection of mechanisms of action.
- Different requirements of circadian genes support and suggest different neuronal mechanisms through which COC and METH induce neuronal plasticity.